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 NEWS  1           Web Page URLs for STN Seminar Schedule - N. America
 NEWS  2  Dec 17   The CA Lexicon available in the CAPLUS and CA files
 NEWS  3  Feb 06   Engineering Information Encompass files have new names
 NEWS  4  Feb 16   TOXLINE no longer being updated
 NEWS  5  Apr 23   Search Derwent WPINDEX by chemical structure
 NEWS  6  Apr 23   PRE-1967 REFERENCES NOW SEARCHABLE IN CAPLUS AND CA
 NEWS  7  May 07   DGENE Reload
 NEWS  8  Jun 20   Published patent applications (A1) are now in USPATFULL
 NEWS  9  JUL 13   New SDI alert frequency now available in Derwent's
                   DWPI and DPCI
 NEWS 10  Aug 23   In-process records and more frequent updates now in
                   MEDLINE
 NEWS 11  Aug 23   PAGE IMAGES FOR 1947-1966 RECORDS IN CAPLUS AND CA
 NEWS 12  Aug 23   Adis Newsletters (ADISNEWS) now available on STN
 NEWS 13  Sep 17   IMSworld Pharmaceutical Company Directory name change
                   to PHARMASEARCH

 NEWS EXPRESS  August 15 CURRENT WINDOWS VERSION IS V6.0c,
               CURRENT MACINTOSH VERSION IS V6.0 (ENG) AND V6.0J (JP),
               AND CURRENT DISCOVER FILE IS DATED 07 AUGUST 2001
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FILE 'HOME' ENTERED AT 14:02:15 ON 25 SEP 2001

=> file medline embase biosis caplus uspatfull
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                     0.15       0.15

FILE 'MEDLINE' ENTERED AT 14:02:40 ON 25 SEP 2001

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```
=> s virus or orthomyxovirus or paramyxovirus
L1     1553025 VIRUS OR ORTHOMYXOVIRUS OR PARAMYXOVIRUS

=> s influenza or parainfluenza or hepatitis or vaccinia or measles or herpes
or respiratory synctium
L2      601467 INFLUENZA OR PARAINFLUENZA OR HEPATITIS OR VACCINIA OR MEASLES
               OR HERPES OR RESPIRATORY SYNCTIUM

=> s l1 and l2
L3      408323 L1 AND L2

=> s sialic acid or hemagglutinin
L4       90830 SIALIC ACID OR HEMAGGLUTININ

=> s l3 and l4
L5       20248 L3 AND L4

=> s skin or topical or derm?
L6     1461266 SKIN OR TOPICAL OR DERM?

=> s l5 and l6
L7        1008 L5 AND L6

=> s immune response or rash or hives
L8      310299 IMMUNE RESPONSE OR RASH OR HIVES

=> s l8 or allergic reasction or allergies or hypersensitiv?
L9      488285 L8 OR ALLERGIC REASCTION OR ALLERGIES OR HYPERSENSITIV?

=> s l7 and l8 and l9
L10         544 L7 AND L8 AND L9

=> s l10 and py not>1999
'1999' NOT A VALID FIELD CODE
'1999' NOT A VALID FIELD CODE
'1999' NOT A VALID FIELD CODE
'1999' NOT A VALID FIELD CODE
'1999' NOT A VALID FIELD CODE
L11           0 L10 AND PY NOT>1999

=> s l10 and py<2000
   2 FILES SEARCHED...
   3 FILES SEARCHED...
L12         307 L10 AND PY<2000

=> dup rem l12
PROCESSING COMPLETED FOR L12
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L13 285 DUP REM L12 (22 DUPLICATES REMOVED)

=> d l13 1-10 kwic

L13 ANSWER 1 OF 285 USPATFULL
PI US 6194173 B1 20010227
WO 9815629 19980416 <--
SUMM . . . present invention features an immunogen comprising a GRP polypeptide in an immunogenic preparation, the immunogen being capable of eliciting an **immune response** specific for a GRP polypeptide; e.g. a humoral response, e.g. an antibody response; e.g. a cellular response. In preferred embodiments,. . .
SUMM . . . or am integral membrane protein mediating cell--cell adhesion, e.g., an ICAM family member. Use of this method is encompassed in **skin** grafting.
DETD . . . promoter region (Bernoist and Chambon, 1981, Nature 290:304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma **virus** (Yamamoto et al., 1980, Cell 22:787-797), the **herpes** thymidine kinase promoter (Wagner et al., 1981, Proc. Natl. Acad. Sci. U.S.A. 78:1441-1445). the regulatory sequences of the metallothionein gene. . .
DETD . . . plasmids, such as pBR322, to facilitate replication and drug resistance selection in both prokaryotic and eukaryotic cells. Alternatively, derivatives of **viruses** such as the bovine papillomavirus (BPV-1), or Epstein-Barr **virus** (pHEBo, pREP-derived and p205) can be used for transient expression of proteins in eukaryotic cells. The various methods employed in. . .
DETD . . . by a target cell). Examples of targeting means include: sterols (e.g. cholesterol), lipids (e.g. a cationic lipid, virosome or liposome), **viruses** (e.g. adenovirus, adeno-associated **virus**, and retrovirus) or target cell specific binding agents (e.g. ligands recognized by target cell specific receptors).
DETD . . . antibodies are to be raised can be incorporated into a fusion gene construct which includes coding sequences for a late **vaccinia virus** structural protein to produce a set of recombinant **viruses** expressing fusion proteins comprising GRP epitopes as part of the virion. It has been demonstrated with the use of immunogenic fusion proteins utilizing the **Hepatitis** B surface antigen fusion proteins that recombinant **Hepatitis** B virions can, be utilized in this role as well. Similarly, chimeric constructs coding for fusion proteins containing a portion. . .
DETD . . . such therapy, the compounds of the invention can be formulated for a variety of loads of administration, including systemic and **topical** or localized administration Techniques and formulations generally may be found in Remmington's Pharmaceutical Sciences, Meade Publishing Co., Easton, Pa. For. . .
DETD . . . In addition, detergents may be used to facilitate permeation. Transmucosal administration may be through nasal sprays or using suppositories. For **topical** administration, the oligomers of the invention are formulated into ointments, salves, gels, or creams as generally known in the art.
DETD . . . precursor of the cell, by way of deliberate genetic manipulation, such as by microinjection or by infection with a recombinant **virus**. The term genetic manipulation does not include classical cross-breeding, or in vitro fertilization, but rather is directed to the introduction. . .
DETD . . . subject GRP modulating agents can either increase or decrease

GRP activity, the agents will be useful for stimulating or suppressing **immune responses**. In one embodiment, GRP modulating agents can be used to modulate inside out signaling of a hematopoietic cell including, but. . .

DETD . . . mellitus, arthritis (including rheumatoid arthritis, juvenile rheumatoid arthritis, osteoarthritis, psoriatic arthritis), multiple sclerosis, myasthenia gravis, systemic lupus erythematosis, autoimmune thyroiditis, **dermatitis** (including atopic **dermatitis** and eczematous **dermatitis**), psoriasis, Sjogren's Syndrome, including keratoconjunctivitis sicca secondary to Sjogren's Syndrome, alopecia areata, allergic responses due to arthropod bite reactions, Crohn's. . . encephalopathy, idiopathic bilateral progressive sensorineural hearing loss, aplastic anemia, pure red cell anemia, idiopathic thrombocytopenia, polychondritis, Wegener's granulomatosis, chronic active **hepatitis**, Stevens-Johnson syndrome, idiopathic sprue, lichen planus, Crohn's disease, Graves ophthalmopathy, sarcoidosis, primary biliary cirrhosis, uveitis posterior, and interstitial lung fibrosis).. . .

DETD . . . Dr. R. Klausner) fused at the carboxy-terminus to a nine amino acid sequence corresponding to the major antigenic determinant of **influenza virus hemagglutinin** were constructed. Sf-9 cells were infected with the recombinant baculovirus and the cells were harvested 3 days later by centrifugation. . .

DETD . . . ARF1. In FIG. 7, panels A-C, isoforms of ARF were tagged with a 9 amino acid sequence corresponding to the **hemagglutinin** antigenic epitope, immunoprecipitated, and incubated in the presence of [.sup.35 S]GTP.gamma.S and 0.15 .mu.M GRP, as indicated. The values are.

. .

L13 ANSWER 2 OF 285 USPATFULL

PI US 6165722 20001226
WO 9820159 19980514 <--

SUMM . . . Vaccinology is based upon the discovery of epitopes within the pathogen of interest which can be used to elicit an **immune response** which can neutralize that pathogen. Once these epitopes have been found, they can be presented to the immune system as. . .

SUMM . . . with a particular pathogen or with an antigen of that pathogen.

For example, sera from human patients immunized against a **hepatitis** B viral antigen, an envelope protein from the **virus**, were used to screen a random library of nonapeptides (peptides of nine amino acids) inserted into the coat protein pVIII.. .

.

SUMM . . . map an antigen is given by Wang et al. [Wang et al., J. Immun. Methods, 1995, 178:1-12] for the bluetongue **virus** outer capsid protein VP5. Bluetongue **virus** infects sheep and cattle. VP5 is a known antigen for this **virus** and is 526 amino acids in length. The VP5 gene was partially digested using DNAase I, an enzyme which cuts. . .

SUMM . . . et al. [Ulmer et al., Curr. Op. Immunol., 1996, 8:531-536], naked DNA vaccines have been shown to be effective against **influenza virus** in animals. A particular bonus of these naked DNA vaccines is that they can elicit cellular as well as antibody. . .

SUMM An example of the importance of discontinuous epitopes is in the study of HIV, or human immunodeficiency **virus**. HIV causes almost invariably fatal disease in humans; so far, no cure or vaccine has been

found. An important step. . .
SUMM . . . the carbohydrates themselves. These interactions are important for a number of biological processes, including lymphocyte migration and
binding of the **hemagglutinin** protein of the human **influenza virus** to erythrocyte glycoproteins, an important step in infection by the **virus**. However, these interactions have typically been difficult to study, because of the difficulty in synthesizing complex carbohydrate ligands. To solve. . .
DETD As an example, consider the production of a complete pepscan of the entire HIV (Human Immunodeficiency **Virus**) genome, which is about 9000 bp. About 3000 peptides of a desired size range would be required in order to. . .
DETD Clearly, a phage display library could be prepared for a number of organisms including, but not limited to **viruses** including but not limited to retrovirus species such as HIV and HTLV, **hepatitis** species such as **Hepatitis** A and **Hepatitis** B, **influenza** species, human papillomavirus, **herpes** species such as **herpes** simplex, RSV (respiratory syncytial **virus**) and cytomegalovirus; bacteria including, but not limited to, Mycobacterium tuberculosis, salmonella, staphylococcus species such as Staph. aureus, and shigella; parasites. . .
DETD . . . the organism to be vaccinated. The first biological unit is typically, but not necessarily, a pathogenic organism, such as a **virus**, bacterium, yeast or parasite. The first biological unit could also be a toxic substance, such as a snake, insect or. . .
DETD Formulations for **topical** administration may include but are not limited to lotions, ointments, gels, creams, suppositories, drops, liquids, sprays and powders. Conventional pharmaceutical. . .
DETD . . . antibodies. Polyclonal antibodies can be prepared by injecting a rabbit, for example, with a substance or substances which provoke an **immune response**. For commercial purposes, a mammal with a larger blood volume can be used, such as a horse for example. Such. . .
DETD . . . cells. Such cells can be prepared from mice cells, for example, or by immortalization of human B-cells by EBV (Epstein-Barr **virus**), for example.
DETD Frequently it is desirable to diagnose the presence of an **immune response** to a particular organism in a tissue or blood sample, or even in vivo. Such detection can be accomplished by. . .
DETD . . . can perform such a diagnosis has many potential biomedical uses, including the detection of organisms including, but not limited to **viruses** including but not limited to retrovirus species such as HIV and HTLV, **hepatitis** species such as **Hepatitis** A and **Hepatitis** B, **influenza** species, human papillomavirus, **herpes** species such as **herpes** simplex, RSV (respiratory syncytial **virus**) and cytomegalovirus; bacteria including, but not limited to, Mycobacterium tuberculosis, salmonella, staphylococcus species such as Staph. aureus, and shigella; parasites. . .

L13 ANSWER 3 OF 285 USPATFULL
PI US 6127170 20001003
WO 9610038 19960404 <--
SUMM . . . genetic information into the genome of the cell. These viral vector systems have relied upon the molecular machinery of the

virus, evolved over time to surmount the significant problems facing a **virus** in attempting to invade, i.e., infect a cell. Despite the efficiency of such viral vectors, however, there has been continued concern regarding the safety of using **viruses**, particularly from the standpoint of undesired side effects. Thus, there has been an ongoing effort to develop non-viral gene delivery. . .

SUMM . . . variety of host cells such as yeast, bacterial or mammalian cells. Genetic material can also be used to provide protective **immune responses** in vivo by injection of DNA that encodes immunogenic proteins, i.e., ones that can stimulate the desired **immune response**. The in vivo introduction of exogenous genetic material into cells also has potential utility in applications for the alleviation, treatment. . .

SUMM . . . recognized that DNA, by itself, injected into various tissues, will enter cells and produce a protein that will elicit an **immune response**. See, e.g., P. Atanasiu et al., Academie des Sciences (Paris) 254, 4228-30 (1962); M. A. Israel et al., J. Virol.. . .

SUMM . . . phosphate groups of the DNA. Orosomucoid is a glycoprotein which is normally present in human serum. Removal of the terminal **sialic acid** (N-acetyl neuraminic acid) from the branched oligosaccharides exposes terminal galactose oligosaccharides, for which hepatocyte receptors have a high affinity, as. . .

SUMM . . . see K. D. Mack, R. Walzem and J. B. Zeldis, Am. J. Med. Sci., 307, 138-143 (1994) or associated with **viruses**. Many **viruses** infect specific cells by receptor mediated binding and insertion of the viral DNA/RNA into the cell; and thus this action of the **virus** is similar to the facilitated entry of DNA described above.

SUMM Synthetic peptides such as the N-terminus region of the **influenza hemagglutinin** protein are known to destabilize membranes and are known as fusogenic peptides. Conjugates containing the **influenza** fusogenic peptide coupled to poly-lysine together with a peptide having a branched tri-lysine amino terminus ligand ending with four galactosyl. . . (1992). These conjugates combine the asialoglycoprotein receptor mediated binding conferred by the tetra-galactose peptide, the endosomal disrupting abilities of the **influenza** fusogenic peptide, and the DNA binding of the poly-lysine. These conjugates deliver DNA into the cell by a combination of receptor mediated uptake and internalization into endosomes. This internalization is followed by disruption of the endosomes by the **influenza** fusogenic peptide to release the DNA into the cytoplasm. In a similar fashion, the **influenza** fusogenic peptide can be attached to poly-lysine and mixed with the transferrin-poly-lysine complex to provide a similar DNA carrier selective. . .

SUMM . . . comprising a) at least one lipophilic long chain alkyl group, b) a fusogenic peptide comprising spike glycoproteins of enveloped animal **viruses**, or c) cholic acid or cholesteryl or derivatives; and optionally 4) one or more receptor specific binding components which are. . .

SUMM . . . comprising i) at least one lipophilic long chain alkyl group, ii) a fusogenic peptide comprising spike glycoproteins of enveloped animal **viruses**, or iii) cholic acid or cholesteryl or derivatives; and optionally 4) one or more receptor specific binding components which are. . .

SUMM . . . retroviral particles. The desired protein may either be a protein which functions within the individual or serves to initiate an **immune response**. The nucleic acid molecule may be administered to the cells of said individual on either an in vivo or ex.

. .
SUMM i) fusogenic peptides comprising spike glycoproteins of enveloped animal
viruses;
SUMM . . . example, where it is desired to transfer nucleic acid molecules
to target cells by injecting them intramuscularly to evoke an **immune response**, it will be found that this transfer can be effected by use of a multifunctional molecular complex of the present. . .
SUMM The fusogenic peptide which functions as an endosome membrane disruption
promoting component, comprises the spike glycoproteins of enveloped animal **viruses** known in the art. Membrane fusion, whether planar or annular, comprises the stages of initial approach, coalescence, and separation. Fusion reactions are rapid, highly specific, and non-leaky. The membrane proteins of enveloped animal **viruses** comprise glycoproteins which span the bilayer of the **virus** membrane and have the bulk of their mass externally, and nonspanning, nonglycosylated proteins associated with the inner bilayer surface. The glycoproteins form radial projections on the surface of the
virus membrane, and these spike glycoproteins play a key role in **virus** entry into host cells. Spike glycoproteins are among the best-characterized **virus** membrane proteins. In cell entry the spike glycoproteins are responsible for attachment of the **virus** particle to the cell surface, and for penetration of the nucleocapsid into the cytosol, where, after endocytosis of the **virus** particle, the spike glycoproteins play a role in fusion with the limiting membrane of the endosome, whereby the nucleocapsid reenters the
cytosol. In some enveloped animal **viruses**, the spike glycoproteins take on a specialized character, e.g., in **orthomyxoviruses**, where one is a neuraminidase and another is a haemagglutinin. All of these fusogenic peptides, in terms of their amino. . .
SUMM Examples of such fusogenic peptides and homologues derived from spike glycoproteins of enveloped **viruses**, include the following peptide sequences reading from N-terminus to C-terminus:
SUMM SEQ ID NO:24: FAGVVLAGAALGVAAAAQI--**measles**;
SUMM SEQ ID NO:25: FAGVVLAGAALGVATAAQI--**measles** (P. L. Yeagle, R. M. Epand, C. D. Richardson and T. D. Flanagan, Biochim. Biophys. Acta, 1065, 49-53 (1991));
SUMM . . . retroviral particles. The desired protein may either be a protein which functions within the individual or serves to initiate an **immune response**.
SUMM . . . be targeted. The genetic material is expressed by the individual's cells and serves as an immunogenic target against which an **immune response** is elicited. The resulting **immune response** is broad based: in addition to a humoral **immune response**, both arms of the cellular **immune response** are elicited. The methods of the present invention are useful for conferring prophylactic and therapeutic
immunity. Thus, a method of. . . an individual suffering from pathogen infection, hyperproliferative disease or autoimmune disease. Thus, the present invention is useful to elicit broad **immune responses** against a target protein, i.e. proteins specifically associated with pathogens or the individual's own "abnormal" cells.
SUMM The present invention is also useful in combating hyperproliferative

diseases and disorders such as cancer, by eliciting an **immune response** against a target protein that is specifically associated with the hyperproliferative cells. The present invention is further useful in combating autoimmune diseases and disorders by eliciting an **immune response** against a target protein that is specifically associated with cells involved in the autoimmune condition.

SUMM . . . peptides and proteins encoded by gene constructs used in the present invention, which either act as target proteins for an **immune response**, or as a therapeutic or compensating protein in gene therapy regimens.

SUMM . . . acid composition that comprises a nucleotide sequence that encodes a target protein, including pharmaceutical preparations useful to invoke a therapeutic **immune response**.

SUMM As used herein, the term "target protein" refers to a protein against which an **immune response** can be elicited. The target protein is an immunogenic protein which shares at least an epitope with a protein from. . . undesirable cell-type, such as a cancer cell or a cell involved in autoimmune disease, against which immunization is required. The **immune response** directed against the target protein will protect the individual against, and treat the individual for, the specific infection or disease. . .

SUMM . . . has a structure which is not identical to an epitope of a protein, but nonetheless invokes a cellular or humoral **immune response** which cross reacts to that protein.

SUMM . . . invention, especially in the production of a genetic vaccine for humans, include but are not limited to, promoters from Simian **Virus** 40 (SV40), Mouse Mammary Tumor **Virus** (MMTV) promoter, Human Immunodeficiency **Virus** (HIV) such as the HIV Long Terminal Repeat (LTR) promoter, Moloney **virus**, ALV, Cytomegalovirus (CMV) such as the CMV immediate early promoter, Epstein Barr **Virus** (EBV), Rous Sarcoma **Virus** (RSV), as well as promoters from human genes such as human Actin, human Myosin, human Hemoglobin, human muscle creatine and. . .

SUMM . . . copies of the construct in the cell. Plasmids pCEP4 and pREP4 from Invitrogen (San Diego, Calif.) contain the Epstein Barr **virus** origin of replication and nuclear antigen EBNA-1 coding region, which produces high copy episomal replication without integration. In aspects of. . .

SUMM . . . nucleic acid composition contains nucleotide sequences that encode a target protein and further include genes for proteins which enhance the **immune response** against such target proteins. Examples of such genes are those which encode cytokines and lymphokines such as .alpha.-interferon, .gamma.-interferon, platelet. .

SUMM . . . for cell destruction, if it is desirable to eliminate the cells receiving the nucleic acid composition for any reason. A **herpes** thymidine kinase (tk) gene in an expressible form can be included in the nucleic acid composition. The drug gangcyclovir can. . .

SUMM . . . a nucleic acid composition into a cell does not constitute introduction of sufficient genetic information for the production of infectious **virus**.

SUMM The present invention may be used to immunize an individual against all pathogens such as **viruses**, prokaryotic and pathogenic eukaryotic organisms such as unicellular pathogenic organisms and multicellular parasites. The present invention is particularly useful to

immunize an individual against those pathogens which infect cells and which are not encapsulated, such as **viruses**, and prokaryotes such as Gonorrhoea, Listeria and Shigella. In addition, the present invention is also useful for immunizing an individual. . . cycle in which they are intracellular pathogens. As used herein, the term "intracellular pathogen" is meant to refer to a **virus** or pathogenic organism that, during at least part of its reproductive or life cycle, exists within a host cell and. . .

SUMM . . . to produce a genetic vaccine to protect against pathogen infection, genetic material which encodes immunogenic proteins against which a protective **immune response** can be mounted, must be included in the nucleic acid composition. Whether the pathogen infects intracellularly, for which the present. . . using two constructs which each contain a different half of the genome which are administered at different sites. Thus, an **immune response** may be invoked against each antigen without the risk of an infectious **virus** being assembled. This allows for the introduction of more than a single antigen target and can eliminate the requirement that. . .

SUMM In accordance with the present invention there is also provided a method of conferring a broad based protective **immune response** against hyperproliferating cells that are characteristic of hyperproliferative diseases, as well as a method of treating individuals suffering from hyperproliferative. . .

SUMM . . . present invention provides a method of treating individuals suffering from autoimmune diseases and disorders by conferring a broad based protective **immune response** against targets that are associated with autoimmunity, including cell receptors and cells which produce "self"-directed antibodies.

SUMM . . . (RA), multiple sclerosis (MS), Sjogren's syndrome, sarcoidosis, insulin dependent diabetes mellitus (IDDM), autoimmune thyroiditis, reactive arthritis, ankylosing spondylitis, scleroderma, polymyositis, **dermatomyositis**, psoriasis, vasculitis, Wegener's granulomatosis, Crohn's disease and ulcerative colitis. Each of these diseases is characterized by T cell receptors that. . . initiate the inflammatory cascade associated with autoimmune diseases. Vaccination against the variable region of the T cells would elicit an **immune response** including CTLs to eliminate those T cells.

SUMM . . . and V.beta.-17. Thus, vaccination with a nucleic acid composition that encodes at least one of these proteins will elicit an **immune response** that will target T cells involved in RA. See: Howell, M. D., et al., 1991 Proc. Natl. Acad. Sci. USA. . .

SUMM . . . and V.beta.-10. Thus, vaccination with a nucleic acid composition that encodes at least one of these proteins will elicit an **immune response** that will target T cells involved in MS. See: Wucherpfennig, K. W., et al., 1990 Science 248:1016-1019; Oksenberg, J. R.,. . .

SUMM . . . and V.alpha.-12. Thus, vaccination with a nucleic acid composition that encodes at least one of these proteins will elicit an **immune response** that will target T cells involved in scleroderma.

SUMM . . . and initiate the inflammatory cascade associated with autoimmune diseases. Vaccination against the variable region of such antibodies would elicit an **immune response** including CTLs to eliminate those B cells that produce the antibody.

SUMM . . . these complexes to all of these tissues. They are particularly useful to deliver the complexes of the present invention to **skin** and muscle cells.

SUMM . . . propel the complexes of the present invention in liquid form, that contains DNA molecules, toward the surface of the individual's **skin**. The liquid is propelled at a sufficient velocity such that upon impact with the **skin**, the liquid penetrates the surface of the **skin**, and permeates the **skin** and muscle tissue therebeneath. Thus, the nucleic acid composition is simultaneously administered intradermally, subcutaneously and intramuscularly. In some embodiments, a. . .

SUMM In some embodiments of the invention, the individual is subject to a single vaccination to produce a full, broad **immune response**. In other embodiments of the invention, the individual is subject to a series of vaccinations to produce a full, broad **immune response**. According to still other embodiments of the invention, at least two and preferably four to five injections are given over. . .

SUMM . . . a complete set of viral genes into the individual without the risk of assembling an infectious viral particle. Thus, an **immune response** against most or all of the **virus** can be invoked in the vaccinated individual. Injection of each inoculant is performed at different sites, preferably at a distance. . .

DETD . . . (Medical) responsible for 50% cases of the common cold.

Etheroviruses: (Medical) includes polioviruses, coxsackieviruses, echoviruses, and human enteroviruses such as **hepatitis** A **virus**.

Apthoviruses: (veterinary) these are the foot and mouth disease **viruses**.

Target antigens: VP1, VP2, VP3, VP4, VPG

Calcivirus Family

Genera: Norwalk Group of **Viruses**: (Medical) these **viruses** are an important causative agent of epidemic gastroenteritis.

Togavirus Family

Genera: Alphaviruses: (Medical and Veterinary) examples include Senilis **viruses**, RossRiver **virus** and Eastern & Western Equine encephalitis.

Reovirus: (Medical) Rubella **virus**.

Flariviridue Family Examples include: (Medical) dengue, yellow fever, Japanese encephalitis, St. Louis encephalitis and tick borne encephalitis **viruses**.

Hepatitis C **Virus**: (Medical) these **viruses** are not placed in a family yet but are believed to be either a togavirus or a flavivirus. Most similarity is with togavirus family.

Coronavirus Family: (Medical and veterinary)

Infectious bronchitis **virus** (poultry)

Porcine transmissible gastroenteric **virus** (pig)

Porcine hemagglutinating encephalomyelitis **virus** (pig)

Feline infectious peritonitis **virus** (cats)

Feline enteric coronavirus (cat)

Canine coronavirus (dog)
The human respiratory coronaviruses cause .about.40
cases of common cold. EX. 224E, 0C43
Note - coronaviruses may cause non-A, B or C
hepatitis
Target antigens: E1 - also called M or matrix protein
E2 - also called S or Spike protein
E3 -. . . Rhabdovirus Family
Genera: Vesiliovirus
Lyssavirus: (medical and veterinary) rabies
Target antigen: G protein
N protein
Filoviridue Family: (Medical) Hemorrhagic fever **viruses** such as Marburg
and
Ebola **virus**
Paramyxovirus Family:
Genera: **Paramyxovirus**: (Medical and Veterinary)
Mumps **virus**, New Castle disease **virus**
(important pathogen in chickens)
Morbillivirus: (Medical and Veterinary)
Measles, canine distemper
Pneuminvirus: (Medical and Veterinary)
Respiratory syncytial **virus**
Orthomyxovirus Family (Medical) The **Influenza virus**
Bungavirus Family
Genera: Bungavirus: (Medical) California encephalitis,
LA Crosse
Phlebovirus: (Medical) Rift Valley Fever
Hantavirus: Puremala is a hemahagin fever
virus
Nairvirus (Veterinary) Nairobi sheep disease
Also many unassigned bungaviruses
Arenavirus Family (Medical) LCM, Lassa fever **virus**
Reovirus Family
Genera: Reovirus: a possible human pathogen
Rotavirus: acute gastroenteritis in children
Orbiviruses: (Medical and Veterinary)
Colorado Tick fever, Lebombo (humans) equine
encephalosis, blue tongue
Retrovirus Family
Sub-Family: Oncorivirinal: (Veterinary) (Medical) feline
leukemia **virus**, HTLVI and HTLVII
Lentivirinal: (Medical and Veterinary) HIV,
feline immunodeficiency **virus**, equine
infectians, anemia **virus**
Spumavirinal
Papovavirus Family
Sub-Family: Polyomaviruses: (Medical) BKU and JCU **viruses**
Sub-Family: Papillomavirus: (Medical) many viral types
associated with cancers or malignant
progression of papilloma
Adenovirus (Medical) EX AD7, ARD., O.B.. . . Sub-Family: Gammaherpesviridue
Genera: Lymphocryptovirus (Medical)
EBV - (Burkitts lympho)
Rhadinovirus
Poxvirus Family
Sub-Family: Chordopoxviridue (Medical - Veterinary)

Genera: Variola (Smaiipox)
Vaccinia (Cowpox)
Parapoxivirus - Veterinary
Auipoxvirus - Veterinary
Capripoxvirus
Leporipoxvirus
Suipoxvirus
Sub-Family: Entemopoxviridue
Hepadnavirus Family **Hepatitis** B **virus**
Unclassified **Hepatitis** delta **virus**

DETD . . . include: actinomycosis; nocardiosis; cryptococcosis, blastomycosis, histopiasmosis and coccidioidomycosis; candidiasis, aspergillosis, and mucormycosis; sporotrichosis; paracoccidiodomycosis, petriellidiosis, torulopsosis, mycetoma and chromomycosis; and **dermatophytosis**. Rickettsial infections include rickettsial and rickettsioses.
Examples of mycoplasma and chlamydial infections include: mycoplasma pneumoniae; lymphogranuloma venereum; psittacosis; and perinatal. . .

DETD . . . ID NOS: 32
- - <210> SEQ ID NO 1
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: Vesicular stomatitis **virus**
- - <400> SEQUENCE: 1
- - Lys Phe Thr Ile Val Phe Pro His Asn Gln Ly - #s. . . 25
- - - - <210> SEQ ID NO 2
<211> LENGTH: 32
<212> TYPE: PRT
<213> ORGANISM: Human immunodeficiency **virus** type - #1
- - <400> SEQUENCE: 2
- - Ala Val Gly Ile Gly Ala Leu Phe Leu Gly. . . 30
- - - - <210> SEQ ID NO 3
<211> LENGTH: 35
<212> TYPE: PRT
<213> ORGANISM: Murine leukemia **virus**
- - <400> SEQUENCE: 3
- - Glu Pro Val Ser Leu Thr Leu Ala Leu Leu Le - #u. . . 35
- - - - <210> SEQ ID NO 4
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Human immunodeficiency **virus** type - #1
- - <400> SEQUENCE: 4
- - Ala Val Gly Ile Gly Ala Leu Phe Leu Gly. . . 20
- - - - <210> SEQ ID NO 5
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Human immunodeficiency **virus** type - #1
- - <400> SEQUENCE: 5
- - Ala Val Gly Ala Ile Gly Ala Leu Phe Leu. . . - - Gly
- - - - <210> SEQ ID NO 6
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: **Influenza virus**
- - <400> SEQUENCE: 6
- - Gly Leu Phe Glu Ala Ile Ala Glu Phe Ile Gl - #u. . . Cys Ala
20

- - - - <210> SEQ ID NO 7
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: **Influenza virus**
- - <400> SEQUENCE: 7
- - Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile Gl - #u. . . 25
- - - - <210> SEQ ID NO 8
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Human immunodeficiency **virus** type - #1
- - <400> SEQUENCE: 8
- - Ala Val Gly Ile Gly Ala Leu Phe Leu Gly. . . Ser
20
- - - - <210> SEQ ID NO 9
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: SV5 **virus**
- - <400> SEQUENCE: 9
- - Phe Ala Gly Val Val Ile Gly Leu Ala Ala Le - #u. . . # 25
- - - - <210> SEQ ID NO 10
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: SFV **virus**
- - <400> SEQUENCE: 10
- - Lys Val Tyr Thr Gly Val Tyr Pro Phe Met Tr - #p. . . Cys Asp
- - - - <210> SEQ ID NO 11
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: PH-30 **virus**
- - <400> SEQUENCE: 11
- - Lys Leu Ile Cys Thr Gly Ile Ser Ser Ile Pr - #o. . . Pro
20
- - - - <210> SEQ ID NO 12
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Sendai **virus**
- - <400> SEQUENCE: 12
- - Phe Phe Gly Ala Val Ile Gly Thr Ile Ala Le - #u. . . Thr
20
- - - - <210> SEQ ID NO 13
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: SV5 **virus**
- - <400> SEQUENCE: 13
- - Phe Ala Gly Val Val Ile Gly Leu Ala Ala Le - #u. . . Thr
20
- - - - <210> SEQ ID NO 14
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: NDV **virus**
- - <400> SEQUENCE: 14
- - Phe Ile Gly Ala Ile Ile Gly Gly Val Ala Le - #u. . . Ile Thr
20
- - - - <210> SEQ ID NO 15
<211> LENGTH: 28
<212> TYPE: PRT
<213> ORGANISM: **Influenza virus**
- - <400> SEQUENCE: 15
- - Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile Gl - #u. . . - #
25

- - - - <210> SEQ ID NO 16
<211> LENGTH: 28
<212> TYPE: PRT
<213> ORGANISM: **Influenza virus**
- - <400> SEQUENCE: 16
- - Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile Gl - #u. . . - #
25
- - - - <210> SEQ ID NO 17
<211> LENGTH: 28
<212> TYPE: PRT
<213> ORGANISM: **Influenza virus**
- - <400> SEQUENCE: 17
- - Gly Phe Phe Gly Ala Ile Ala Gly Phe Leu Gl - #u. . . # 25
- - - - <210> SEQ ID NO 18
<211> LENGTH: 30
<212> TYPE: PRT
<213> ORGANISM: MMTV **virus**
- - <400> SEQUENCE: 18
- - Phe Val Ala Ala Ile Ile Leu Gly Ile Ser Al - #a. . . # 30
- - - - <210> SEQ ID NO 19
<211> LENGTH: 53
<212> TYPE: PRT
<213> ORGANISM: MoMLV **virus**
- - <400> SEQUENCE: 19
- - Glu Pro Val Ser Leu Thr Leu Ala Leu Leu Le - #u. . . Ser
50
- - - - <210> SEQ ID NO 20
<211> LENGTH: 53
<212> TYPE: PRT
<213> ORGANISM: F-MuLV **virus**
- - <400> SEQUENCE: 20
- - Glu Pro Val Ser Leu Thr Leu Ala Leu Leu Le - #u. . . Ser
50
- - - - <210> SEQ ID NO 21
<211> LENGTH: 53
<212> TYPE: PRT
<213> ORGANISM: AKV **virus**
- - <400> SEQUENCE: 21
- - Glu Pro Val Ser Leu Thr Leu Ala Leu Leu Le - #u. . . Ser
50
- - - - <210> SEQ ID NO 22
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: SFV **virus**
- - <400> SEQUENCE: 22
- - Asp Tyr Gln Cys Lys Val Tyr Thr Gly Val Ty - #r. . . # 25
- - - - <210> SEQ ID NO 23
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Sindbis **virus**
- - <400> SEQUENCE: 23
- - Asp Tyr Thr Cys Lys Val Phe Gly Gly Val Ty - #r. . . - #
25
- - - - <210> SEQ ID NO 24
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: **Measles virus**
- - <400> SEQUENCE: 24
- - Phe Ala Gly Val Val Leu Ala Gly Ala Ala Le - #u. . . Ala Gln Ile
- - - - <210> SEQ ID NO 25

<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: **Measles virus**
- - <400> SEQUENCE: 25
- - Phe Ala Gly Val Val Leu Ala Gly Ala Ala Le - #u. . . Ala Gln Ile
- - - - <210> SEQ ID NO 26
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: **Influenza virus**
- - <400> SEQUENCE: 26
- - Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile Gl - #u. . . Gly Cys
20
- - - - <210> SEQ ID NO 27
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: **Influenza virus**
- - <400> SEQUENCE: 27
- - Gly Leu Phe Gly Ala Ile Ala - #Gly Phe Ile Glu Asn. . . - # 20
- - - - <210> SEQ ID NO 28
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: **Influenza virus**
- - <400> SEQUENCE: 28
- - Gly Ile Phe Gly Ala Ile Ala - #Gly Phe Ile Glu Asn. . . - # 20
- - - - <210> SEQ ID NO 29
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: **Influenza virus**
- - <400> SEQUENCE: 29
- - Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile Gl - #u. . . Asp Gly
20
- - - - <210> SEQ ID NO 30
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: **Influenza virus**
- - <400> SEQUENCE: 30
- - Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile Gl - #u. . . Asp Gly
20
- - - - <210> SEQ ID NO 31
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: **Influenza virus**
- - <400> SEQUENCE: 31
- - Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile Gl - #u. . . Asp Gly
20
- - - - <210> SEQ ID NO 32
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: **Influenza virus**
- - <400> SEQUENCE: 32
- - Gly Phe Phe Gly Ala Ile Ala Gly Phe Leu Gl - #u. . .

CLM What is claimed is:

. . . absent; and R.sup.4 is independently selected from the group consisting of: (i) fusogenic peptides comprising spike glycoproteins of enveloped animal **viruses**; (ii) cholic acid derivatives of the formula (2): ##STR5## where: represents a bond of unspecified stereochemistry; --- represents a single. . .

. . . absent; and R.sup.4 is independently selected from the group consisting of: (i) fusogenic peptides comprising spike glycoproteins of enveloped animal **viruses**; (ii) cholic acid derivatives of the

formula (2): ##STR7## where: represents a bond of unspecified stereochemistry; --- represents a single. . .
. . . absent; and R.sup.4 is independently selected from the group consisting of: (i) fusogenic peptides comprising spike glycoproteins of enveloped animal **viruses**; (ii) cholic acid derivatives of the formula (2): ##STR9## where: represents a bond of unspecified stereochemistry; --- represents a single. . .
. . . absent; and R.sup.4 is independently selected from the group consisting of: (i) fusogenic peptides comprising spike glycoproteins of enveloped animal **viruses**; (ii) cholic acid derivatives of the formula (2): ##STR11## where: represents a bond of unspecified stereochemistry; --- represents a single. . .
. . . absent; and R.sup.4 is independently selected from the group consisting of: (i) fusogenic peptides comprising spike glycoproteins of enveloped animal **viruses**; (ii) cholic acid derivatives of the formula (2): ##STR13## where: represents a bond of unspecified stereochemistry; --- represents a single. . .

L13 ANSWER 4 OF 285 USPATFULL
TI Recombinant swinepox **virus**
PI US 6127163 20001003
WO 9503070 19950202
AB The present invention relates to a recombinant swinepox **virus** capable of replication comprising foreign DNA inserted into a site in the swinepox viral DNA which is not essential for replication of the swinepox **virus**. The invention further relates to homology vectors which produce recombinant swinepox **viruses** by inserting foreign DNA into swinepox viral DNA
SUMM Swinepox **virus** (SPV) belongs to the family Poxviridae. **Viruses** belonging to this group are large, double-stranded DNA **viruses** that characteristically develop in the cytoplasm of the host cell. SPV is the only member of the genus Suipoxvirus. Several features distinguish SPV from other poxviruses. SPV exhibits species specificity (18) compared to other poxviruses such as **vaccinia** which exhibit a broad host range. SPV infection of tissue culture cell lines also differs dramatically from other poxviruses (24). It has also been demonstrated that SPV does not exhibit antigenic cross-reactivity with **vaccinia virus** and shows no gross detectable homology at the DNA level with the ortho, lepori, avi or entomopox **virus** groups (24). Accordingly, what is known and described in the prior art regarding other poxviruses does not pertain a priori to swinepox **virus**.
SUMM SPV is only mildly pathogenic, being characterized by a self-limiting infection with lesions detected only in the **skin** and regional lymph nodes. Although the SPV infection is quite limited, pigs which have recovered from SPV are refractory to. . .
SUMM . . . SPV support this rationale: SPV is only mildly pathogenic in swine, SPV is species specific, and SPV elicits a protective **immune response**. Accordingly, SPV is an excellent candidate for a viral vector delivery system, having little intrinsic risk which must be balanced. . .
SUMM Among the poxviruses, five (**vaccinia**, fowlpox, canarypox, pigeon, and raccoon pox) have been engineered, previous to this disclosure, to contain foreign DNA sequences. **Vaccinia virus** has been used extensively to vector foreign genes (25) and is the subject of U.S. Pat. Nos. 4,603,112 and 4,722,848.. . . 89/03429, and PCT WO 89/12684. Raccoon pox (10) and Canarypox (31) have been utilized to express antigens from the rabies **virus**. These examples of insertions of foreign genes into poxviruses do not include an example from the genus Suipoxvirus. Thus, they do not teach methods

to genetically engineer swinepox **viruses**, that is, where to make insertions and how to get expression in swinepox **virus**.

SUMM The idea of using live **viruses** as delivery systems for antigens has a very long history going back to the first live **virus** vaccines. The antigens delivered were not foreign but were naturally expressed by the live **virus** in the vaccines. The use of **viruses** to deliver foreign antigens in the modern sense became obvious with the recombinant **vaccinia virus** studies. The **vaccinia virus** was the vector and various antigens from other disease causing **viruses** were the foreign antigens, and the vaccine was created by genetic engineering. While the concept became obvious with these disclosures, what was not obvious was the answer to a more practical question of what makes the best candidate **virus** vector. In answering this question, details of the pathogenicity of the **virus**, its site of replication, the kind of **immune response** it elicits, the potential it has to express foreign antigens, its suitability for genetic engineering, its probability of being licensed. . .

SUMM The prior art relating to the use of poxviruses to deliver therapeutic agents relates to the use of a **vaccinia virus** to deliver interleukin-2 (12). In this case, although the interleukin-2 had an attenuating effect on the **vaccinia** vector, the host did not demonstrate any therapeutic benefit.

SUMM . . . delivered by a viral vector of the present invention must be a biological molecule that is a by-product of swinepox **virus** replication. This limits the therapeutic agent in the first analysis to either DNA, RNA or protein. There are examples of. . .

SUMM The invention provides a recombinant swinepox **virus** capable of replication which comprises swinepox viral DNA and foreign DNA encoding RNA which does not naturally occur in an animal into which the recombinant swinepox **virus** is introduced. The foreign DNA is inserted into the swinepox viral DNA at a site which is not essential for replication of the swinepox **virus** and is under the control of a promoter.

SUMM This invention provides a homology vector for producing a recombinant swinepox **virus** by inserting foreign DNA into the genomic DNA of a swinepox **virus** which comprises a double-stranded DNA molecule. This molecule consists essentially of double-stranded foreign DNA encoding RNA which does not naturally occur in an animal into which the recombinant swinepox **virus** is introduced. At one end of this foreign DNA is double-stranded swinepox viral DNA homologous to genomic DNA located at one side of a site on the genomic DNA which is not essential for replication of the swinepox **virus**. At the other end of the foreign DNA is double-stranded swinepox viral DNA homologous to genomic DNA located at the. . .

DRWD FIGS. 3A, 3B, and 3C show the homology which exists between the 515.85.1 ORF and the **Vaccinia virus** 01L ORF. FIG. 3A shows two maps: The first line of FIG. 3A is a restriction map of the SPV. . .

DRWD . . . in brackets [ ] indicate the remnants of sites which were destroyed during construction. The following abbreviations are used, swinepox **virus** (SPV), early promoter 1 (EPI), late promoter 2 (LP2), lactose operon Z gene (lacZ), and Escherichia coli (E. coli).

DRWD . . . in brackets [ ] indicate the remnants of sites which were destroyed during construction. The following abbreviations are used, swinepox **virus** (SPV), pseudorabies **virus** (PRV), g50 (gpD), glycoprotein 63 (gp63), early promoter 1 (EP1), late promoter 1 (LP1) (SEQ ID NO: 46), late promoter. . .

DRWD . . . of lysates from recombinant SPV infected cells with anti-serum to PRV. Lanes (A) uninfected Vero cell lysate, (B) S-PRV-000 (pseudorabies **virus** S62/26) infected cell lysate, (C) pre-stained molecular weight markers, (D) uninfected EMSK cell lysate, (E) S-SPV-000 infected cell lysate, (F). . .

DRWD DNA sequence of NDV **Hemagglutinin**-Neuraminidase gene (HN) (SEQ ID NO: 29). The sequence of 1907 base pairs of the NDV HN cDNA clone are shown.. . .

DRWD . . . in brackets [ ] indicate the remnants of sites which were destroyed during construction. The following abbreviations are used, swinepox **virus** (SPV), Newcastle Disease **virus** (NDV), **hemagglutinin**-neuraminidase (HN), early promoter 1 (EP1), late promoter 1 (LP1), late promoter 2 (LP2), lactose operon Z gene (lacZ), and Escherichia. . .

DRWD FIGS. 9A, 9B, and 9C show a detailed description of Swinepox **Virus** S-SPV-010 and the DNA insertion in Homology Vector 561-36.26. FIG. 9A contains a diagram showing the orientation of DNA fragments. . . in brackets, [ ], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used:

swinepox **virus** (SPV), Escherichia coli (E. coli), thymidine kinase (TK), pox synthetic late promoter 1 (LP1), base pairs (BP).

DRWD FIGS. 10A, 10B, 10C, and 10D show a detailed description of Swinepox **Virus** S-SPV-011 and the DNA insertion in Homology Vector 570-91.21. FIG. 10A contains a diagram showing the orientation of DNA fragments. . . in brackets, [ ], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used:

swinepox **virus** (SPV), pseudorabies **virus** (PRV), Escherichia coli (E. coli), pox synthetic late promoter 1 (LP1), pox synthetic early promoter 2 (EP2) (SEQ ID NO:. . .

DRWD FIGS. 11, 11B, 11C and 11D show a detailed description of Swinepox **Virus** S-SPV-012 and the DNA insertion in Homology Vector 570-91.41. FIG. 11A contains a diagram showing the orientation of DNA fragments. . . sites brackets, [ ], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used: swinepox **virus** (SPV), pseudorabies **virus** (PRV), Escherichia coli (E. coli), pox synthetic late promoter 1 (LP1), pox synthetic early promoter 1 late promoter 2 (EP1LP2). . .

DRWD FIGS. 12, 12B, 12C and 12D show a detailed description of Swinepox **Virus** S-PRV-013 and the DNA insertion in Homology Vector 570-91.64. FIG. 12A contains a diagram showing the orientation of DNA fragments. . . in brackets, [ ], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used:

swinepox **virus** (SPV), pseudorabies **virus** (PRV), Escherichia coli (E. coli), pox synthetic late promoter 1 (LP1), pox synthetic late promoter 2 early promoter 2 (LP2EP2). . .

DRWD FIGS. 13A, 13B, 13C and 13D show a detailed description of Swinepox **Virus** S-PRV-014 and the DNA insertion in Homology Vector 599-65.25. FIG. 13A contains a diagram showing the orientation of DNA fragments. . .

DRWD swinepox **virus** (SPV), infectious laryngotracheitis **virus** (ILT), Escherichia coli (E. coli), pox synthetic late promoter 1 (LP1), pox synthetic early promoter 1 late promoter 2

(EP1LP2),. . .

DRWD FIGS. 14A, 14B, 14C, and 14D show a derailed description of Swinepox **Virus** S-SPV-016 and the DNA insertion in Homology Vector 624-20.1C. FIG. 14A contains a diagram showing the orientation of DNA fragments. . . in brackets, [ ], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used: swinepox **virus** (SPV), infectious laryngotracheitis **virus** (ILT), Escherichia coli (E. coli), pox synthetic late promoter 1 (LP1), pox synthetic late promoter 2 early promoter 2 (LP2EP2),. . .

DRWD FIGS. 15A, 15B, 15C and 15D show a detailed description of Swinepox **Virus** S-SPV-017 and the DNA insertion in Homology Vector 614-83.18. FIG. 15A contains a diagram showing the orientation of DNA fragments. . . in brackets, [ ], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used: swinepox **virus** (SPV), infectious bovine rhinotracheitis **virus** (IBR), Escherichia coli (E. coli), pox synthetic late promoter 1 (LP1), pox synthetic late promoter 2 early promoter 2 (LP2EP2),. . .

DRWD Map showing the 3,628 base pair BglII to HindIII swinepox **virus** DNA fragment inserted into homology vector 515-85.1.

DRWD FIGS. 18A, 18B, 18C and 18D show a detailed description of Swinepox **Virus** S-SPV-034 and the DNA insertion in Homology Vector 723-59A9.22. FIG. 18A contains a diagram showing the orientation of DNA fragments. . . in brackets, [ ], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used: swinepox **virus** (SPV), equine **influenza virus** (EIV), Escherichia coli (E. coli), pox synthetic late promoter 1 (LP1), pox synthetic late promoter 2 early promoter 2 (LP2EP2),. . .

DRWD FIGS. 19A, 19B, 19C and 19D show a detailed description of Swinepox **Virus** S-SPV-015 and the DNA insertion in Homology Vector 727-54.60. FIG. 19A contains a diagram showing the orientation of DNA fragments. . . in brackets, [ ], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used: swinepox **virus** (SPV), pseudorabies **virus** (PRV), Escherichia coli (E. coli), pox synthetic late promoter 1 (LP1), pox synthetic late promoter 2 early promoter 2 (LP2EP2),. . .

DRWD FIGS. 20A, 20B, 20C, and 20D show a detailed description of Swinepox **Virus** S-SPV-031 and the DNA insertion in Homology Vector 727-67.18. FIG. 20A contains a diagram showing the orientation of DNA fragments. .

DRWD . . . in brackets, [ ], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used: swinepox **virus** (SPV), Escherichia coli (E. coli), pox synthetic late promoter 1 (LP1), pox synthetic early promoter 1 late promoter 2 (EP1LP2),. . .

DRWD FIGS. 21A, 21B, 21C and 21D show a detailed description of Swinepox **Virus** S-SPV-033 and the DNA insertion in Homology Vector 732-18.4. FIG. 21A contains a diagram showing the orientation of DNA fragments. . . in brackets, [ ], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used:

swinepox **virus** (SPV), Escherichia coli (E. coli), pox synthetic late promoter 1 (LP1), pox synthetic late promoter 2 early promoter 2 (LP2EP2), equine **influenza virus** (EIV), neuraminidase (NA), Alaska (AK), polymerase chain reaction (PCR), base pairs (BP).

DRWD FIGS. 22A, 22B and 22C show a detailed description of Swinepox **Virus** S-SPV-036 and the DNA insertion in Homology Vector 741-80.3. FIG. 22A contains a diagram showing the orientation of DNA fragments. . . in brackets, [ ], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used: swinepox **virus** (SPV), pseudorabies **virus** (PRV), Escherichia coli (E. coli), human cytomegalovirus immediate early (HCMV IE), pox synthetic late promoter 1 (LP1), pox synthetic late. . .

DRWD FIGS. 23A, 23B, 23C and 23D show a detailed description of Swinepox **Virus** S-SPV-035 and the DNA insertion in Homology Vector 741-84.14. FIG. 23A contains a diagram showing the orientation of DNA fragments. . . in brackets, [ ], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used: swinepox **virus** (SPV), pseudorabies **virus** (PRV), Escherichia coli (E. coli), pox synthetic late promoter 1 (LP1), pox synthetic late promoter 2 early promoter 2 (LP2EP2),. . .

DRWD FIGS. 24A, 24B, 24C and 24D show a detailed description of Swinepox **Virus** S-SPV-038 and the DNA insertion in Homology Vector 744-34. FIG. 24A contains a diagram showing the orientation of DNA fragments. .

. . . . in brackets, [ ], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used: swinepox **virus** (SPV), equine herpesvirus type 1 (EHV-1) , Escherichia coli (E. coli) , pox synthetic late promoter 1 (LPs), pox synthetic. . .

DRWD FIGS. 25A, 25B, 25C, and 25D show a detailed description of Swinepox **Virus** S-SPV-039 and the DNA insertion in Homology Vector 744-38. FIG. 25A contains a diagram showing the orientation of DNA fragments. .

. in brackets, [ ], indicate the remnants of sites which are destroyed during construction. The following abbreviations are used: swinepox **virus** (SPV), equine herpesvirus type 1 (EHV-1), Escherichia coli (E. coli), pox synthetic late promoter 1 (LP1), pox synthetic late promoter. . .

DETD The present invention provides a recombinant swinepox **virus** (SPV) capable of replication in an animal into which the recombinant swinepox **virus** is introduced which comprises swinepox viral DNA and foreign DNA encoding RNA which does not naturally occur in the animal into which the recombinant swinepox **virus** is introduced, the foreign DNA being inserted into the swinepox viral DNA at an insertion site which is not essential for replication of the swinepox **virus** and being under the control of a promoter.

DETD For purposes of this invention , "a recombinant swinepox **virus** capable of replication" is a live swinepox **virus** which has been generated by the recombinant methods well known to those of skill in the art, e.g., the methods. . . RECOMBINANT SPV in Materials and Methods and has not had genetic material essential for the replication of the recombinant swinepox **virus** deleted.

DETD For purposes of this invention, "an insertion site which is not essential for replication of the swinepox **virus**" is a location

in the swinepox viral genome where a sequence of DNA is not necessary for viral replication, for. . .

DETD . . . reading frame encoding swinepox thymidine kinase. Preferably, the insertion site is the NdeI restriction endonuclease site located within the swinepox **virus** thymidine kinase gene.

DETD The invention further provides a recombinant swinepox **virus** capable of replication which contains a foreign DNA encoding a polypeptide which is a detectable marker. Preferably the detectable marker. . . the AccI restriction endonuclease site located within the HindIII M fragment of the swinepox viral DNA. Preferably, this recombinant swinepox **virus** is designated S-SPV-003 (ATCC Accession No. VR 2335). The S-SPV-003 swinepox **virus** has been deposited pursuant to the Budapest Treaty on the International Deposit of Microorganisms for the Purposes of Patent Procedure. . .

DETD The invention further provides a recombinant swinepox **virus** capable of replication which contains foreign DNA encoding an antigenic polypeptide which is or is from pseudorabies **virus** (PRV) g50 (gpD), pseudorabies **virus** (PRV) II (gpB), Pseudorabies **virus** (PRV) gIII (gpC), pseudorabies **virus** (PRV) glycoprotein H, pseudorabies **virus** (PRV) glycoprotein E, Transmissible gastroenteritis (TGE) glycoprotein 195, Transmissible gastroenteritis (TGE) matrix protein, swine rotavirus glycoprotein 38, swine parvovirus capsid protein, Serpulina hydodysenteriae protective antigen, Bovine Viral Diarrhea (BVD) glycoprotein 55, Newcastle Disease **Virus** (NDV) **hemagglutinin**-neuraminidase, swine flu **hemagglutinin** or swine flu neuraminidase. Preferably, the antigenic polypeptide is Pseudorabies **Virus** (PRV) g50 (gpD). Preferably, the antigenic protein is Newcastle Disease **Virus** (NDV) **hemagglutinin**-neuraminidase.

DETD The invention further provides a recombinant swinepox **virus** capable of replication which contains foreign DNA encoding an antigenic polypeptide which is or is from Serpulina hyodysenteriae, Foot and Mouth Disease **Virus**, Hog Cholera **Virus**, Swine **Influenza Virus**, African Swine Fever **Virus** or Mycoplasma hyopneumoniae.

DETD The invention further provides for a recombinant swinepox **virus** capable of replication which contains foreign DNA encoding pseudorabies **virus** (PRV) g50 (gpD). This recombinant swinepox **virus** can be further engineered to contain foreign DNA encoding a detectable marker, such as E. coli B-galactosidase. A preferred site. . . coli B-galactosidase is the AccI site within the HindIII M fragment of the swinepox viral DNA. Preferably, this recombinant swinepox **virus** is designated S-SPV-008 (ATCC Accession No. VR 2339). The S-SPV-008 swinepox **virus** has been deposited pursuant to the Budapest Treaty on the International Deposit of Microorganisms for the Purposes of Patent Procedure. . .

DETD The invention further provides for a recombinant swinepox **virus** capable of replication which contains foreign DNA encoding pseudorabies **virus** (PRV) gIII (gpC). This recombinant swinepox **virus** can also be further engineered to contain foreign DNA encoding a detectable marker, such as E. coli B-galactosidase. A preferred. . . coli B-galactosidase is the AccI site within the HindIII M fragment of the swinepox viral DNA. Preferably, this recombinant swinepox **virus** is designated S-SPV-011, S-SPV-012, or S-SPV-013. The swinepox **virus** designated S-SPV-013 has been deposited pursuant to the Budapest Treaty on the International Deposit of Microorganisms for the Purposes of. . .

DETD The invention further provides for a recombinant swinepox **virus**

capable of replication which contains foreign DNA encoding pseudorabies **virus** (PRV) gII (gpB). This recombinant swinepox **virus** can also be further engineered to contain foreign DNA encoding a detectable marker, such as E. coli B-galactosidase. A preferred. . . coli B-galactosidase is the AccI site within the HindIII M fragment of the swinepox viral DNA. Preferably, this recombinant swinepox **virus** is designated S-SPV-015. The S-SPV-015 swinepox **virus** has been deposited on Jul. 22, 1994 pursuant to the Budapest Treaty on the International Deposit of Microorganisms for the. . .

DETD The invention further provides for a recombinant swinepox **virus** capable of replication which contains foreign DNA encoding pseudorabies **virus** (PRV) g50 (gpD) and foreign DNA encoding pseudorabies **virus** (PRV) gIII (gpC). This recombinant swinepox **virus** can also be further engineered to contain foreign DNA encoding a detectable marker, such as E. coli B-galactosidase. A preferred. . .

DETD The invention further provides for a recombinant swinepox **virus** capable of replication which contains foreign DNA encoding pseudorabies **virus** (PRV) g50 (gpD) and foreign DNA encoding pseudorabies **virus** (PRV) gII (gpB). This recombinant swinepox **virus** can also be further engineered to contain Foreign DNA encoding a detectable marker, such as E. coli B-galactosidase. A preferred. . .

DETD The invention further provides for a recombinant swinepox **virus** capable of replication which contains foreign DNA encoding pseudorabies **virus** (PRV) gIII (gpC) and foreign DNA encoding pseudorabies **virus** (PRV) gII (gpB). This recombinant swinepox **virus** can also be further engineered to contain foreign DNA encoding a detectable marker, such as E. coli B-galactosidase. A preferred. . .

DETD The invention further provides for a recombinant swinepox **virus** capable of replication which contains foreign DNA encoding pseudorabies **virus** (PRV) g50 (gpD), foreign DNA encoding pseudorabies **virus** (PRV) gIII (gpC), and foreign DNA encoding pseudorabies **virus** (PRV) gII (gpB). This recombinant swinepox **virus** can also be further engineered to contain foreign DNA encoding a detectable marker, such as E. coli B-galactosidase.

DETD The invention further provides for a recombinant swinepox **virus** capable of replication which contains foreign DNA encoding RNA encoding the antigenic polypeptide Newcastle Disease **Virus** (NDV) **hemagglutinin**-neuraminidase further comprising foreign DNA encoding a polypeptide which is a detectable marker. Preferably, this recombinant swinepox **virus** is designated S-SPV-009 (ATCC Accession No. VR 2344). The S-SPV-009 swinepox **virus** has been deposited pursuant to the Budapest Treaty on the International Deposit of Microorganisms for the Purposes of Patent Procedure. . .

DETD The present invention further provides a recombinant swinepox **virus** which comprises a foreign DNA sequence inserted into a non-essential site of the swinepox genome, wherein the foreign DNA sequence encodes an antigenic polypeptide derived from infectious bovine rhinotracheitis **virus** and is capable of being expressed in a host infected by the recombinant swinepox **virus**. Examples of such antigenic polypeptide are infectious bovine rhinotracheitis **virus** glycoprotein E and glycoprotein G. Preferred embodiment of this invention are recombinant swinepox **viruses** designated S-SPV-017 and S-SPV-019.

DETD The present invention further provides a recombinant swinepox **virus** which comprises a foreign DNA sequence inserted into a

non-essential site of the swinepox genome, wherein the foreign DNA sequence encodes an antigenic polypeptide derived from infectious laryngotracheitis **virus** and is capable of being expressed in a host infected by the recombinant swinepox **virus**. Examples of such antigenic polypeptide are infectious laryngotracheitis **virus** glycoprotein G and glycoprotein I. Preferred embodiment of this invention are recombinant swinepox **viruses** designated S-SPV-014 and S-SPV-016.

DETD The present invention further provides a recombinant swinepox **virus** which comprises a foreign DNA sequence inserted into a non-essential site of the swinepox genome, wherein the foreign DNA sequence. . . polypeptide derived from a human pathogen and is capable of being expressed in a host infected by the recombinant swinepox **virus**.

DETD For example, the antigenic polypeptide of a human pathogen is derived from human herpesvirus, **herpes** simplex **virus**-1, **herpes** simplex **virus**-2, human cytomegalovirus, Epstein-Barr **virus**, Varicell-Zoster **virus**, human herpesvirus-6, human herpesvirus-7, human **influenza**, human immunodeficiency **virus**, rabies **virus**, **measles virus**, **hepatitis** B **virus** and **hepatitis** C **virus**. Furthermore, the antigenic polypeptide of a human pathogen may be associated with malaria or malignant tumor from the group conisting. . .

DETD In one embodiment of the invention, a recombinant swinepox **virus** contains the foreign DNA sequence encoding **hepatitis** B **virus** core protein. Preferably, such **virus** recombinant **virus** is designated S-SPV-031.

DETD The present invention further provides a recombinant swinepox **virus** which comprises a foreign DNA sequence inserted into a non-essential site of the swinepox genome, wherein the foreign DNA sequence encodes a cytokine capable of stimulating an immune in a host infected by the recombinant swinepox **virus** and is capable of being expressed in the host infected.

DETD In one embodiment of the invention, a recombinant swinepox **virus** contains a foreign DNA sequence encoding human interleukin-2. Preferably, such recombinant **virus** is designated S-SPV-035.

DETD The present invention further provides a recombinant swinepox **virus** which comprises a foreign DNA sequence inserted into a non-essential site of the swinepox genome, wherein the foreign DNA sequence. . . polypeptide derived from an equine pathogen and is capable of being expressed in a host infected by the recombinant swinepox **virus**.

DETD The antigenic polypeptide of an equine pathogen can derived from equine **influenza virus** or equine herpesvirus. Examples of such antigenic polypeptide are equine **influenza virus** type A/Alaska 91 neuraminidase, equine **influenza virus** type A/Prague 56 neuraminidase, equine **influenza virus** type A/Miami 63 neuraminidase, equine **influenza virus** type A/Kentucky 81 neuraminidaseequine herpesvirus type 1 glycoprotein B, and equine herpesvirus type 1 glycoprotein D. Preferred embodiments of such recombinant **virus** are designated S-SPV-033, S-SPV-034, S-SPV-038, and S-SPV-039.

DETD The present invention further provides a recombinant swinepox **virus** which comprises a foreign DNA sequence inserted into a non-essential site of the swinepox genome, wherein the foreign DNA sequence encodes an antigenic polypeptide derived from bovine respiratory syncytial **virus** or bovine **parainfluenza virus**, and is capable of being expressed in a host infected by the recombinant swinepox **virus**.

DETD For example, the antigenic polypeptide of derived from bovine respiratory syncytial **virus** equine pathogen can derived from equine **influenza virus** is bovine respiratory syncytial **virus** attachment protein (BRSV G), bovine respiratory syncytial **virus** fusion protein (BRSV F), bovine respiratory syncytial **virus** nucleocapsid protein (BRSV N), bovine **parainfluenza virus** type 3 fusion protein, and the bovine **parainfluenza virus** type 3 **hemagglutinin** neuraminidase.

DETD Preferred embodiments of a recombinant **virus** containing a foreign DNA encoding an antigenic polypeptide from a bovine respiratory syncytial **virus** are designated S-SPV-020, S-SPV-029, and S-SPV-030.

DETD And a preferred embodiment of a recombinant **virus** containing a foreign DNA encoding an antigenic polypeptide from a bovine **parainfluenza virus** are designated S-SPV-028.

DETD The present invention further provides a recombinant swinepox **virus** which comprises a foreign DNA sequence inserted into a non-essential site of the swinepox genome, wherein the foreign DNA sequence encodes bovine viral diarrhea **virus** glycoprotein 48 or glycoprotein 53, and wherein the foreign DNA sequence is capable of being expressed in a host infected by the recombinant swinepox **virus**. Preferred embodiments of such **virus** are designated S-SPV-032 and S-SPV-040.

DETD The present invention further provides a recombinant swinepox **virus** which comprises a foreign DNA sequence inserted into a non-essential site of the swinepox genome, wherein the foreign DNA sequence encodes an antigenic polypeptide derived from infectious bursal disease **virus** and wherein the foreign DNA sequence is capable of being expressed in a host infected by the recombinant swinepox **virus**. Examples of such antigenic polypeptide are infectious bursal disease **virus** polyprotein and VP2. Preferred embodiments of such **virus** are designated S-SPV-026 and S-SPV-027.

DETD The invention provides for a homology vector for producing a recombinant swinepox **virus** by inserting foreign DNA into the genomic DNA of a swinepox **virus**. The homology vector comprises a double-stranded DNA molecule consisting essentially of a double-stranded foreign DNA encoding RNA which does not naturally occur in an animal into which the recombinant swinepox **virus** is introduced, with at one end of the foreign DNA, double-stranded swinepox viral DNA homologous to genomic DNA located at one side of a site on the genomic DNA which is not essential for replication of the swinepox **virus** , and at the other end of the foreign DNA, double-stranded swinepox viral DNA homologous to genomic DNA located at the. . .

DETD In one embodiment, the polypeptide is antigenic in the animal. Preferably, the antigenic polypeptide is or is from pseudorabies **virus** (PRV) g50 (gpD), pseudorabies **virus** (PRV) gII (gpB), Pseudorabies **virus** (PRV) gIII (gpC), Pseudorabies **virus** (PRV) glycoprotein H, Transmissible gastroenteritis (TGE) glycoprotein 195, Transmissible gastroenteritis (TGE) matrix protein, swine rotavirus glycoprotein 38, swine parvovirus capsid protein, Serpulina hydodysenteriae protective antigen, Bovine Viral Diarrhea (BVD) glycoprotein 55, Newcastle Disease **Virus** (NDV) **hemagglutinin**-neuraminidase, swine flu **hemagglutinin** or swine flu neuraminidase. Preferably, the antigenic polypeptide is or is from Serpulina hyodesenteriae, Foot and Mouth Disease **Virus**

, Hog Cholera **Virus**, Swine **Influenza Virus**
, African Swine Fever **Virus** or Mycoplasma hyopneumoniae.

DETD For example, the antigenic polypeptide of a human pathogen is derived from human herpesvirus, **herpes** simplex **virus**-1, **herpes** simplex **virus**-2, human cytomegalovirus, Epstein-Barr **virus**, Varicell-Zoster **virus**, human herpesvirus-6, human herpesvirus-7, human **influenza**, human immunodeficiency **virus**, rabies **virus**, **measles virus**, **hepatitis** B **virus** and **hepatitis** C **virus**. Furthermore, the antigenic polypeptide of a human pathogen may be associated with malaria or malignant tumor from the group conisting. . .

DETD . . . the present invention, the double stranded foreign DNA sequence in the homology vector encodes a cytokine capable of stimulating human **immune response**. For example, the cytokine can be, but not limited to, interleukin-2, interleukin-6, interleukin-12, interferons, granulocyte-macrophage colony stimulating factors, and interleukin. . .

DETD The antigenic polypeptide of an equine pathogen can derived from equine **influenza virus** or equine herpesvirus. Examples of such antigenic polypeptide are equine **influenza virus** type A/Alaska 91 neuraminidase, equine **influenza virus** type A/Prague 56 neuraminidase, equine **influenza virus** type A/Miami 63 neuraminidase, equine **influenza virus** type A/Kentucky 81 neuraminidaseequine herpesvirus type 1 glycoprotein B, and equine herpesvirus type 1 glycoprotein D.

DETD . . . invention, the double stranded foreign DNA sequence of the homology vector encodes an antigenic polypeptide derived from bovine respiratory syncytial **virus** or bovine **parainfluenza virus**.

DETD For example, the antigenic polypeptide of derived from bovine respiratory syncytial **virus** equine pathogen can derived from equine **influenza virus** is bovine respiratory syncytial **virus** attachment protein (BRSV G), bovine respiratory syncytial **virus** fusion protein (BRSV F), bovine respiratory syncytial **virus** nucleocapsid protein (BRSV N), bovine **parainfluenza virus** type 3 fusion protein, and the bovine **parainfluenza virus** type 3 **hemagglutinin** neuraminidase.

DETD . . . invention, the double stranded foreign DNA sequence of the homology vector encodes an antigenic polypeptide derived from infectious bursal disease **virus**. Examples of such antigenic polypeptide are infectious bursal disease **virus** polyprotein and infectious bursal disease **virus** VP2.

DETD . . . is homologous to genomic DNA present within the larger HindIII to BglII subfragment of the HindIII M fragment of swinepox **virus**. Preferably, the double-stranded swinepox viral DNA is homologous to genomic DNA present within the open reading frame contained in this. . .

DETD . . . "homology vector" is a plasmid constructed to insert foreign DNA in a specific site on the genome of a swinepox **virus**.

DETD The invention further provides a vaccine which comprises an effective immunizing amount of a recombinant swinepox **virus** of the present invention and a suitable carrier.

DETD Suitable carriers for the pseudorabies **virus** are well known in the art and include proteins, sugars, etc. One example of such a suitable carrier is a. . .

DETD For purposes of this invention, an "effective immunizing amount" of the

recombinant swinepox **virus** of the present invention is within the range of 10.sup.3 to 10.sup.9 PFU/dose.

DETD . . . a human, swine, bovine, equine, caprine or ovine. For purposes of this invention, this includes immunizing the animal against the **virus** or **viruses** which cause the disease or diseases pseudorabies, transmissible gastroenteritis, swine rotavirus, swine parvovirus, Serpulina hyodysenteriae, bovine viral diarrhea, Newcastle disease,. . .

DETD . . . the swine has been vaccinated with the vaccine of the present invention, particularly the embodiment which contains the recombinant swinepox **virus** S-SPV-008 (ATCC Accession No. VR 2339), or is infected with a naturally-occurring, wild-type pseudorabies **virus**. This method comprises obtaining from the swine to be tested a sample of a suitable body fluid, detecting in the sample the presence of antibodies to pseudorabies **virus**, the absence of such antibodies indicating that the swine has been neither vaccinated nor infected, and for the swine in which antibodies to pseudorabies **virus** are present, detecting in the sample the absence of antibodies to pseudorabies **virus** antigens which are normally present in the body fluid of a swine infected by the naturally-occurring pseudorabies **virus** but which are not present in a vaccinated swine indicating that the swine was vaccinated and is not infected.

DETD The present invention also provides a host cell infected with a recombinant swinepox **virus** capable of replication. In one embodiment, the host cell is a mammalian cell. Preferably, the mammalian cell is a Vero. . .

DETD Methods for constructing, selecting and purifying recombinant swinepox **viruses** described above are detailed below in Materials and Methods.

DETD PREPARATION OF SWINEPOX **VIRUS** STOCK SAMPLES. Swinepox **virus** (SPV) samples were prepared by infecting embryonic swine kidney (EMSK) cells, ESK-4 cells, PK-15 cells or Vero cells at a. . . harvested and frozen in a 50 ml conical screw cap tube at -70.degree. C.

Upon thawing at 37.degree. C., the **virus** stock was aliquoted into 1.0 ml vials and refrozen at -70.degree. C. The titers were usually about 10.sup.6 PFU/ml.

DETD PREPARATION OF SPV DNA. For swinepox **virus** DNA isolation, a confluent monolayer of EMSK cells in a T175 cm.sup.2 flask was infected at a multiplicity of 0.1. . .

DETD . . . VERO for PRV) in a 25 cm.sup.2 flask or a 60 mm petri dish was infected with 100 .mu.l of **virus** sample. After cytopathic effect was complete, the medium and cells were harvested and the cells were pelleted at 3000 rpm. . .

DETD HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. This method relies upon the homologous recombination between the swinepox **virus** DNA and the plasmid homology vector DNA which occurs in the tissue culture cells containing both swinepox **virus** DNA and transfected plasmid homology vector. For homologous recombination to occur, the monolayers of EMSK cells are infected with S-SPV-001. . .

DETD . . . cap tube and mixed gently. The mixture was then incubated for 15-20 minutes at room temperature. During this time, the **virus** inoculum was removed from the 6 cm plates and the cell monolayers washed once with EMSK negative medium. Three ml. . . complete medium. The

cells were incubated at 37.degree. C. in 5% CO.sub.2 for 3-7 days until cytopathic effect from the **virus** was 80-100%. **Virus** was harvested as described above for the preparation of **virus** stocks. This stock was referred to as a transfection stock and was subsequently screened for recombinant **virus** by the BLUOGAL SCREEN FOR RECOMBINANT SWINEPOX **VIRUS** OR CPRG SCREEN FOR RECOMBINANT SWINEPOX **VIRUS**.

DETD . . . SPV EXPRESSING B-GALACTOSIDASE (BLUOGAL AND CPRG ASSAYS). When the E. coli .beta.-galactosidase (lacZ) marker gene was incorporated into a recombinant **virus** the plaques containing the recombinants were visualized by one of two simple methods. In the first method, the chemical Bluogal.TM.. . . The red plaques were then picked onto fresh cells (EMSK) and purified by further red plaque isolation. In both cases **viruses** were typically purified with three rounds of plaque purification.

DETD . . . FOREIGN GENE EXPRESSION IN RECOMBINANT SPV USING BLACK PLAQUE ASSAYS. To analyze expression of foreign antigens expressed by recombinant swinepox **viruses**, monolayers of EMSK cells were infected with recombinant SPV, overlayed with nutrient agarose media and incubated for 6-7 days at. . .

DETD . . . We chose to design three promoter cassettes LP1, EP1 and LP2 based on promoters that have been defined in the **vaccinia virus** (1, 7 and 8). Each cassette was designed to contain the DNA sequences defined in **vaccinia** flanked by restriction sites which could be used to combine the cassettes in any order or combination. Initiator methionines were. . .

DETD VACCINATION STUDIES IN SWINE USING RECOMBINANT SWINEPOX **VIRUS** CONTAINING PSEUDORABIES **VIRUS** GLYCOPROTEIN GENES: Young weaned pigs from pseudorabies-free herd are used to test the efficacy of the recombinant swinepox **virus** containing one or more of the pseudorabies **virus** glycoprotein genes (SPV/PRV). The piglets are inoculated intradermally or orally about 10.sup.3 to 10.sup.7 plaque forming units (PFU) of the recombinant SPV/PRV **viruses**.

DETD Immunity is determined by measuring PRV serum antibody levels and by challenging the vaccinated pigs with virulent strain of pseudorabies **virus**. Three to four weeks post-vaccination, both vaccinated and non-vaccinated groups of pigs are challenged with virulent strain of pseudorabies **virus** (VDL4892). Post challenge, the pigs are observed daily for 14 days for clinical signs of pseudorabies.

DETD . . . foreign DNA insert at the AccI site, is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a **virus** containing the foreign DNA will result. A restriction map of the DNA insert in homology vector 515-85.1 is given in. . .

DETD . . . fragment of SPV DNA. When this plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a **virus** containing DNA coding for the marker gene will result. Note that the .beta.-galactosidase (lacZ) marker gene is under the control. . .

DETD . . . fragment of SPV DNA. When this plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a **virus** containing DNA coding for the foreign genes will result. Note that the .beta.-galactosidase (lacZ) marker gene is under the control. . .

DETD . . . fragment of SPV DNA. When this plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a

virus containing DNA coding for the foreign genes will result. Note that the .beta.-galactosidase (lacZ) marker gene is under the control. . .

DETD . . . fragment of SPV DNA. When this plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a **virus** containing DNA coding for the foreign genes will result. Note that the .beta.-galactosidase (lacZ) marker gene is under the control. . .

DETD . . . fragment of SPV DNA. When this plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a **virus** containing DNA coding for the foreign genes will result. Note that the .beta.-galactosidase (lacZ) marker gene is under the control. . .

DETD . . . the purpose of inserting foreign DNA into SPV. It incorporates an E.coli .beta.-galactosidase (lacZ) marker gene and the Newcastle Disease **Virus** (NDV) **hemagglutinin**-Neuraminidase (HN) gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 2149 base pair fragment of SPV. . . fragment of SPV DNA. When this plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a **virus** containing DNA coding for the foreign genes will result. Note that the .beta.-galactosidase (lacZ) marker gene is under the control. . .

DETD . . . fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a **virus** containing DNA coding for the foreign genes will result. Note that the B-galactosidase (lacZ) marker gene is under the control. . . with a template consisting of a 2.6 kb Sst I to Asp718I subfragment of a 5.1 kbAsp718I fragment of ILT **virus** genome. The first primer 91.13 (5'-CCGAATTCCGGCTTCAGTAACATAGGATCG-3') (SEQ ID NO: 81) sits down on the ILT gpG sequence at amino acid. . .

DETD . . . fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a **virus** containing DNA coding for the foreign genes will result. Note that the B-galactosidase (lacZ) marker gene is under the control. . .

DETD . . . fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a **virus** containing DNA coding for the foreign genes will result. Note that the B-galactosidase (lacZ) marker gene is under the control. . . gpG gene. In the PCR cloning procedure, the primers described below were used with a template consisting of the IBR-000 **virus** (Cooper strain). The first primer 106.9 (5'-ATGAATTCCCCTGCCGCCCGGACCGGCACC-3') (SEQ ID NO. 87) sits down on the IBR gpG sequence at amino. . .

DETD . . . flanked by SPV DNA. When this plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a **virus** containing DNA coding for the foreign genes will result. Note that the B-galactosidase (lacZ) marker gene is under the control. . . BamHI ends. In the PCR cloning procedure, the primers described below were used with a template consisting of the IBR-000 **VIRUS** (Cooper strain). The first primer 4/93.17DR (5'-CTGGTTCGGCCCAGAATTCTATGGGTCTCGCGCGGCTCGTGG-3' (SEQ ID NO. 89) sits down on the IBR gpE gene at amino. . .

DETD . . . fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a **virus** containing the DNA coding for the foreign genes results. Note that the B- galactosidase (lacz) marker gene is under the. . .

DETD . . . foreign DNA insert at the NdeI site, is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a **virus** containing the foreign DNA will result. Plasmid 520-90.15 was constructed utilizing standard recombinant DNA techniques (22 and 30), by joining. . .

DETD . . . of inserting foreign DNA into SPV. It incorporates an E. coli .beta.-galactosidase (lacZ) marker gene and the infectious bovine rhinotracheitis **virus** (IBRV) gE gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of. . . of SPV DNA. When the plasmid is used is according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a **virus** containing DNA coding for the foreign genes will result. Note that the .beta.-galactosidase (lacZ) marker gene is under the control. . . gE gene. In the PCR cloning procedure, the primers described below were used with a template consisting of the IBR-000 **virus** (Cooper strain) (44). The first primer 2/94.5DR (5'-CTGGTTCGGCCCAGAATTCGATGCAACCCACCGCGCCGCCCCG-3') (SEQ ID NO. 116) sits down on the IBR gpE gene at. . .

DETD . . . was used to insert foreign DNA into SPV. It incorporates an E. coli .beta.-galactosidase (lacZ) marker gene and the equine **influenza virus** NA PR/56 gene flanked by SPV DNA. When this plasmid was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a **virus** containing DNA coding for the foreign genes results. Note that the .beta.-galactosidase (lacZ) marker gene is under the control of. . . restriction sub-fragment of the SPV HindIII fragment M (23). Fragment 2 is the NA gene coding region from the equine **Influenza** A/Prague/56 (serotype 1 (N7) **virus**) cloned as an approximately 1450 base pair BamHI fragment-utilizing the following primers 5'-GGGATCCATGAATCCTAATCAAAAACTCTTT-3' (SEQ ID NO: 118) for cDNA priming and combined with 5'-GGGATCCTTACGAAAAGTATTTAATTTGTGC-3' (SEQ ID NO: 119) for PCR. (see CLONING OF EQUINE **INFLUENZA VIRUS HEMAGGLUTININ** AND NEURAMINIDASE GENES). Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11).. . .

DETD . . . the purpose of inserting foreign DNA into SPV. It incorporates an E. coli .beta.-galactosidase (lacz) marker gene and the pseudorabies **virus** (PRV) gII (gpB) gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment. . . fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a **virus** containing DNA coding for the foreign genes will result. Note that the .beta.-galactosidase (lacZ) marker gene is under the control. . .

DETD . . . for the purpose of inserting foreign DNA into SPV. It incorporates an E. coli .beta.-galactosidase (lacZ) marker gene and the **hepatitis** B **virus** core antigen gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of. . . fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a **virus** containing DNA coding for the foreign genes will result. Note that the .beta.-galactosidase (lacZ) marker gene is under the control of a synthetic late pox promoter (LP1), and the **hepatitis** B core antigen gene is under the control of a synthetic early/late pox promoter (EP1LP2). A detailed description of the. . . is an approximately 589 base pair fragment with BamHI and

EcoRI restriction sites at the ends. This fragment contains the **hepatitis** B core antigen coding sequences (amino acids 25-212) (Ref. 45, 50). Fragment 4 is an approximately 2149 base pair AccI. . .

DETD CLONING OF EQUINE **INFLUENZA VIRUS** **HEMAGGLUTININ** AND NEURAMINIDASE GENES. The equine **influenza virus hemagglutinin** (HA) and Neuraminidase (NA) genes may be cloned essentially as described by Katz et al. (42) for the HA gene of human **influenza virus** . Viral RNA prepared from **virus** grown in MDBK cells (for **Influenza** A/equine/Alaska/91 and **Influenza** A/equine/Miami/63) and MDCK cells (for **Influenza** A/equine/Prague/56 and **Influenza** A/equine/Kentucky/81) is first converted to cDNA utilizing an oligo nucleotide primer specific for the target gene. The cDNA is then. . . oligo nucleotide primers will be required for each coding region. The HA gene coding regions from the serotype 2 (H3) **viruses** (**Influenza** A/equine/Miami/63, **Influenza** A/equine/Kentucky/81, and **Influenza** A/equine/Alaska/91) would be cloned utilizing the following primers 5'-GGAGGCCTTCATGACAGACAACCATTATTTTGATACTACTGA-3' (SEQ ID NO: 120) for cDNA priming and combined with 5'-GAAGGCCTTCTCAAATGCAAATGTTGCATCTGATGTTGCC-3' (SEQ ID NO: 121) for PCR. The HA gene coding region from the serotype 1 (H7) **virus** (**Influenza** A/equine/Prague/56) would be cloned utilizing the following primers 5'-GGGATCCATGAACACTCAAATTCTAATATTAG-3' (SEQ ID NO: 122) for cDNA priming and combined with 5'-GGGATCCTTATATACAAATAGTGCACCGC A-3' (SEQ ID NO: 123) for PCR. The NA gene coding regions from the serotype 2 (N8) **viruses** (**Influenza** A/equine/Miami/63, **Influenza** A/equine/Kentucky/81, and **Influenza** A/equine/Alaska/91) would be cloned utilizing the following primers 5'-GGGTCGACATGAATCCAAATCAAAAGATAA-3' (SEQ ID NO: 124) for cDNA priming and combined with 5'-GGGTCGACTTACATCTTATCGATGTCAAA-3' (SEQ ID NO: 125) for PCR. The NA gene coding region from the serotype 1 (N7) **virus** (**Influenza**/A/equine/Prague/56) would be cloned utilizing the following primers 5'-GGGATCCATGAATCCTAATCAAAAACTCTT T-3' (SEQ ID NO: 118) for cDNA priming and combined with 5'-GGGATCCTTACGAAAAGTATTTAATTTGTGC-3' (SEQ. . . general strategy may be used to clone the coding regions of HA and NA genes from other strains of equine **influenza** A **virus**. The EIV HA or NA genes are cloned as a blunt ended SalI or BamHI fragment into a blunt ended. . .

DETD . . . was used to insert foreign DNA into SPV. It incorporates an E. coli .beta.-galactosidase (lacZ) marker gene and the equine **influenza virus** AK/91 NA gene flanked by SPV DNA. When this plasmic was used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a **virus** containing DNA coding for the foreign genes results. Note that the .beta.-galactosidase (lacZ) marker gene is under the control of. . . restriction sub-fragment of the SPV HindIII fragment M (23). Fragment 2 is the NA gene coding region from the equine **Influenza** A/Alaska/91 (serotype 2 (N8) **virus**) cloned as an approximately 1450 base pair SalI fragment utilizing the following primers 5'-GGGTCGACATGAATCCAAATCAAAAGATAA-3' (SEQ ID NO: 124) for cDNA priming and combined with 5'-GGGTCGACTTACATCTTATCGATGTCAAA-3' (SEQ ID NO: 125) for PCR (see CLONING OF EQUINE **INFLUENZA VIRUS** **HEMAGGLUTININ** AND NEURAMINIDASE GENES). Fragment 3 is an

approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11).. . .

DETD . . . fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a **virus** containing DNA coding for the foreign genes will result. Note that the .beta.-galactosidase (lacZ) marker gene is under the control. . .

DETD . . . fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a **virus** containing DNA coding for the foreign genes will result. Note that the .beta.-galactosidase (lacZ) marker gene is under the control. . .

DETD . . . fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a **virus** containing DNA coding for the foreign genes will result. Note that the .beta.-galactosidase (lacZ) marker gene is under the control. . .

DETD . . . fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a **virus** containing DNA coding for the foreign genes will result. Note that the .beta.-galactosidase (lacZ) marker gene is under the control. . .

DETD CLONING OF **PARAINFLUENZA**-3 **VIRUS** FUSION AND **HEMAGGLUTININ** GENES. The **parainfluenza**-3 **virus** fusion (F) and **hemagglutinin** (HN) genes were cloned by a PCR CLONING procedure essentially as described by Katz et al. (42) for the HA gene of human **influenza**. Viral RNA prepared from bovine PI-3 **virus** grown in Madin-Darby bovine kidney (MDBK) cells was first converted to cDNA utilizing an oligonucleotide primer specific for the target. . .

DETD CLONING OF BOVINE VIRAL DIARRHEA **VIRUS** gp48 and gp53 GENES. The bovine viral diarrhea gp48 and gp53 genes were cloned by a PCR CLONING procedure essentially as described by Katz et al. (42) for the HA gene of human **influenza**. Viral RNA prepared from BVD **virus** Singer strain grown in Madin-Darby bovine kidney (MDBK) cells was first converted to cDNA utilizing an oligonucleotide primer specific for. . .

DETD CLONING OF BOVINE RESPIRATORY SYNCYTIAL **VIRUS** FUSION, NUCLEOCAPSID AND GLYCOPROTEIN GENES. The bovine respiratory syncytial **virus** fusion (F), nucleocapsid (N), and glycoprotein (G) genes were cloned by a PCR CLONING procedure essentially as described by Katz et al. (42) for the HA gene of human **influenza**. Viral RNA prepared from BRSV **virus** grown in bovine nasal turbinate (BT) cells was first converted to cDNA utilizing an oligonucleotide primer specific for the target. . .

DETD . . . of inserting foreign DNA into SPV. It incorporates an E. coli .beta.-galactosidase (lacz) marker gene and the infectious bursal disease **virus** (IBDV) polyprotein gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of. . . fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a **virus** containing DNA coding for the foreign genes will result. Note that the .beta.-galactosidase (lacZ) marker gene is under the control. . .

DETD . . . of inserting foreign DNA into SPV. It incorporates an E. coli .beta.-galactosidase (lacZ) marker gene and the infectious bursal disease **virus** (IBDV) VP2 gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 1484 base pair fragment of. . .

fragment of SPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV, a **virus** containing DNA coding for the foreign genes will result. Note that the .beta.-galactosidase (lacZ) marker gene is under the control. . .

DETD . . . (AccI) be in a region non-essential to the replication of the SPV and that the site be flanked with swinepox **virus** DNA appropriate for mediating homologous recombination between **virus** and plasmid DNAs. We have demonstrated that the AccI site in homology vector 515-85.1 may be used to insert foreign. . .

DETD . . . potential sites. The resulting plasmids were utilized in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The generation of recombinant **virus** was determined by the SCREEN FOR RECOMBINANT SPV EXPRESSING .beta.-GALACTOSIDASE ASSAYS. Four of the six sites were found to generate recombinant **virus**, however the ability of each of these **viruses** to be purified away from the parental SPV varied greatly. In one case **virus** could not be purified above the level of 1%, in another case **virus** could not be purified above the level of 50%, and in a third case **virus** could not be purified above the level of 90%. The inability to purify these **viruses** indicates instability at the insertion site. This makes the corresponding sites inappropriate for insertion of foreign DNA. However the insertion at one site, the AccI site of Homology vector 515-85.1, resulted in a **virus** which was easily purified to 100% (see example 2), clearly defining an appropriate site for the insertion of foreign DNA.

DETD . . . first region codes for an open reading frame (ORF) which shows homology to amino acids 1 to 115 of the **vaccinia virus** (VV) O1L open reading frame identified by Goebel et al, 1990 (see FIGS. 3A, 3B and 3C) . The sequence. . . second region codes for an open reading frame which shows homology to amino acids 568 to 666 of the same **vaccinia virus** O1L open reading frame (see FIGS. 3A, 3B and 3C). These data suggest that the AccI site interrupts the presumptive. . . of certain eukaryotic transcriptional regulatory proteins, however they indicate that it is not known whether this gene is essential for **virus** replication. The DNA sequence located upstream of the VV 01L-like ORF (see FIG. 2A) would be expected to contain a. . .

DETD S-SPV-003 is a swinepox **virus** that expresses a foreign gene. The gene for E.coli .beta.-galactosidase (lacZ gene) was inserted into the SPV 515-85.1 ORF. The. . .

DETD S-SPV-003 was derived from S-SPV-001 (Kasza strain). This was accomplished utilizing the homology vector 520-17.5 (see Materials and Methods) and **virus** S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING .beta.-GALACTOSIDASE (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant **virus** designated S-SPV-003. This **virus** was assayed for .beta.-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the **virus** was pure, stable and expressing the foreign gene. The assays described here were carried out in VERO cells as well. . .

DETD S-SPV-008 is a swinepox **virus** that expresses at least two foreign genes. The gene for E. coli .beta.-galactosidase (lacz gene) and

the gene for pseudorabies **virus** (PRV) g50 (gpD) (26) were inserted into the SPV 515-85.1 ORF. The lacZ gene is under the control of a. . .

DETD S-SPV-008 was derived from S-SPV-001 (Kasza strain). This was accomplished utilizing the homology vector 538-46.16 (see Materials and Methods) and **virus** S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING .beta.-GALACTOSIDASE (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant **virus** designated S-SPV-008. This **virus** was assayed for .beta.-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the **virus** was pure, stable and expressing the marker gene.

DETD . . . plaques and not with S-SPV-009 negative control plaques. All S-SPV-008 observed plaques reacted with the swine antiserum indicating that the **virus** was stably expressing the PRV foreign gene. The black plaque assay was also performed on unfixed monolayers. The SPV plaques. . .

DETD . . . behind several synthetic pox promoters. The techniques utilized for the creation of S-SPV-008 will be used to create recombinant swinepox **viruses** expressing all four of these PRV glycoprotein genes. Such recombinant swinepox **viruses** will be useful as vaccines against PRV disease. Since the PRV vaccines described here do not express PRV gpX or. . .

DETD S-SPV-011 is a swinepox **virus** that expresses at least two foreign genes. The gene for E. coli B-galactosidase (lacZ) and the gene for pseudorabies **virus** gIII (gpC) were inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site) of the. . .

DETD S-SPV-011 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 570-91.21 (see Materials and Methods) and **virus** S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING B-GALACTOSIDASE (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant **virus** designated S-SPV-011. This **virus** was assayed for B-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the **virus** was pure, stable, and expressing the foreign gene.

DETD . . . and not with S-SPV-001 negative control plaques. All S-SPV-011 observed plaques reacted with the swine anti-PRV serum indicating that the **virus** was stably expressing the PRV foreign gene. The assays described here were carried out in EMSK cells, indicating that EMSK. . .

DETD Recombinant-expressed PRV gIII (gpC) has been shown to elicit a significant **immune response** in mice and swine (37, 38). Furthermore, when gIII (gpC) is coexpressed with gII (gpB) or g50 (gpD), significant protection. . .

DETD S-SPV-012 is a swinepox **virus** that expresses at least two foreign genes. The gene for E. coli B-galactosidase (lacz) and the gene for pseudorabies **virus** gIII (gpC) were inserted into the

unique PstI restriction site (PstI linkers inserted into a unique AccI site) of the. . .

DETD S-SPV-012 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 570-91.41 (see Materials and Methods) and **virus** S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING B-GALACTOSIDASE (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant **virus** designated S-SPV-012. This **virus** was assayed for B-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the **virus** was pure, stable, and expressing the foreign gene.

DETD . . . and not with S-SPV-001 negative control plaques. All S-SPV-012 observed plaques reacted with the swine anti-PRV serum, indicating that the **virus** was stably expressing the PRV foreign gene. The assays described here were carried out in EMSK and VERO cells, indicating. . .

DETD Recombinant-expressed PRV gIII (gpC) has been shown to elicit a significant **immune response** in mice and swine (37, 38). Furthermore, when gIII (gpC) is coexpressed with gII (gpB) or g50 (gpD), significant protection. . .

DETD S-SPV-013 is a swinepox **virus** that expresses at least two foreign genes. The gene for E. coli B-galactosidase (lacZ) and the gene for pseudorabies **virus** gill (gpC) were inserted into the unique PstI restriction site (PstI linkers inserted into a unique AccI site) of the. . .

DETD S-SPV-013 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 570-91.64 (see Materials and Methods) and **virus** S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING B-GALACTOSIDASE (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant **virus** designated S-SPV-013. This **virus** was assayed for B-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the **virus** was pure, stable, and expressing the foreign gene.

DETD . . . and not with S-SPV-001 negative control plaques. All S-SPV-013 observed plaques reacted with the swine anti-PRV serum indicating that the **virus** was stably expressing the PRV foreign gene. The assays described here were carried out in EMSK and VERO cells, indicating. . .

DETD Recombinant-expressed PRV gIII (gpC) has been shown to elicit a significant **immune response** in mice and swine (37, 38). Furthermore, when gIII (gpC) is coexpressed with gII (gpB) or g50 (gpD), significant protection. . .

DETD Protection against Aujeszky's disease using recombinant swinepox **virus** vaccines S-SPV-008 and S-SPV-013.

DETD A vaccine containing S-SPV-008 and S-SPV-013 (1 x 106PFU/ml) (2 ml of a 1:1 mixture of the two **viruses**) was given to two groups of pigs (5 pigs per group) by intradermal inoculation or by oral/pharyngeal spray. A control. . .

DETD S-SPV-015 is a swinepox **virus** that expresses at least two foreign genes. The gene for E. coli .beta.-galactosidase (lacZ) and the gene for pseudorabies **virus** (PRV) gII (gpB) were inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique AccI. . .

DETD . . . was derived from S-SPV-001 (Kasza Strain) . This was accomplished utilizing the homology vector 727-54.60 (see Materials and Methods) and **virus** S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING .beta.-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant **virus** designated S-SPV-015. This **virus** was assayed for .beta.-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the **virus** was pure, stable, and expressing the foreign gene.

DETD . . . S-SPV-015 plaques and not with S-SPV-001 negative control plaques. All S-SPV-015 observed plaques reacted with the antiserum indicating that the **virus** was stably expressing the PRV foreign gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4. . .

DETD S-SPV-015 is useful as a vaccine in swine against pseudorabies **virus**. A superior vaccine is formulated by combining S-SPV-008 (PRV g50), S-SPV-013 (PRV gIII), and S-SPV-015 for protection against pseudorabies in. . .

DETD Recombinant swinepox **virus** expressing more than one pseudorabies **virus** (PRV) glycoproteins, which can elicit production of neutralizing antibodies against pseudorabies **virus** , is constructed in order to obtain a recombinant swinepox **virus** with enhanced ability to protect against PRV infection than that which can be obtained by using a recombinant swinepox **virus** expressing only one of those PRV glycoproteins.

DETD There are several examples of such recombinant swinepox **virus** expressing more than one PRV glycoproteins: a recombinant swinepox **virus** expressing PRV g50 (gpD) and gIII (gpC), a recombinant swinepox **virus** expressing PRV g50 (gpD) and gII (gpB); a recombinant swinepox **virus** expressing PRV gII (gpB) and gIII (gpC); and a recombinant swinepox **virus** expressing PRV g50 (gpD), gIII (gpC) and gII (gpB). Each of the **viruses** cited above is also engineered to contain and express E. coli B-galactosidase (lac Z) gene, which will facilitate the cloning of the recombinant swinepox **virus**.

DETD Listed below are three examples of a recombinant swinepox **virus** expressing PRV g50 (gpD), PRV gIII (gpC), PRV gII (gpB) and E. coli B-galactosidase (lacZ):

DETD a) Recombinant swinepox **virus** containing and expressing PRV g50 (gpD) gene, PRV gIII (gpC) gene, PRV gII (gpB) gene and lacZ gene. All four genes are inserted into the unique AccI restriction endonuclease site within the HindIII M fragment of the swinepox **virus** genome. PRV g50 (gpD) gene is under the control of a synthetic early/late promoter (EP1LP2), PRV gIII (gpC) gene is. . .

DETD b) Recombinant swinepox **virus** containing and expressing PRV g50 (gpD) gene, PRV gIII (gpC) gene, PRV gII (gpB) gene and lacz gene. All four genes are inserted into the unique AccI restriction endonuclease site within the HindIII M fragment of the swinepox **virus** genome. PRV g50 (gpD) gene is under the control of a

synthetic early/late promoter (EP1LP2), PRV gIII (gpC) gene is. . .

DETD c) Recombinant swinepox **virus** containing and expressing PRV g50 (gpD) gene, PRV gIII (gpC) gene, PRV gII (gpB) gene and lacZ gene. All four genes are inserted into the unique AccI restriction endonuclease site within the HindIII M fragment of the swinepox **virus** genome. PRV g50 (gpD) gene is under the control of a synthetic early/late promoter (EP1LP2), PRV gIII (gpC) gene is. . .

DETD S-SPV-009 is a swinepox **virus** that expresses at least two foreign genes. The gene for E. coli .beta.-galactosidase (lacZ gene) and the gene for Newcastle's Disease **virus hemagglutinin** (HN) gene were inserted into the SPV 515-85.1 ORF. The lacZ gene is under the control of a synthetic late. . .

DETD S-SPV-009 was derived from S-SPV-001 (Kasza strain). This was accomplished utilizing the homology vector 538-46.26 (see Materials and Methods) and **virus** S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING .beta.-GALACTOSIDASE (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant **virus** designated S-SPV-009. This **virus** was assayed for .beta.-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the **virus** was pure, stable and expressing the marker gene.

DETD . . . plaques and not with S-SPV-008 negative control plaques. All S-SPV-009 observed plaques reacted with the swine antiserum indicating that the **virus** was stably expressing the NDV foreign gene. S-SPV-009 has been deposited with the ATCC under Accession No. VR 2344).

DETD S-SPV-014 is a swinepox **virus** that expresses at least two foreign genes. The gene for E. coli B-galactosidase (lacZ) and the gene for infectious laryngotracheitis **virus** glycoprotein G (ILT gpG) were inserted into the SPV 570-33.32 ORF (a unique PstI site has replaced the unique AccI. . .

DETD S-SPV-014 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 599-65.25 (see Materials and Methods) and **virus** S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING B-GALACTOSIDASE (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant **virus** designated S-SPV-014. This **virus** was assayed for B-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the **virus** was pure, stable, and expressing the foreign gene. The assays described here were carried out in ESK-4 cells, indicating that. . .

DETD This **virus** is used as an expression vector for expressing ILT glycoprotein G (gpG). Such ILT gpG is used as an antigen to identify antibodies directed against the wild-type ILT **virus** as opposed to antibodies directed against gpG deleted ILT **viruses**. This **virus** is also used as an antigen for the production of ILT gpG specific monoclonal antibodies. Such antibodies are useful in the development of diagnostic tests specific for the ILT gpG protein.

Monoclonal antibodies are generated in mice utilizing this **virus** according to the PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials & Methods).

DETD S-SPV-016 is a swinepox **virus** that expresses at least two foreign genes. The gene for E. coli B-galactosidase (lacZ) and the gene for infectious laryngotracheitis **virus** glycoproteinI (ILT gpI) were inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique AccI. . .

DETD S-SPV-016 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 624-20.1C (see Materials and Methods) and **virus** S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING B-GALACTOSIDASE (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant **virus** designated S-SPV-016. This **virus** was assayed for B-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, al; plaques observed were blue indicating that the **virus** was pure, stable, and expressing the foreign gene.

DETD . . . plaques and not with S-SPV-017 negative control plaques. All S-SPV-016 observed plaques reacted with the chicken antiserum indicating that the **virus** was stably expressing the ILT foreign gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4. . .

DETD This **virus** is used as an expression vector for expressing ILT glycoprotein I (gpI). Such ILT gpI is used as an antigen to identify antibodies directed against the wild-type ILT **virus** as opposed to antibodies directed against gpI deleted ILT **viruses**. This **virus** is also used as an antigen for the production of ILT gpI specific monoclonal antibodies. Such antibodies are useful in the development of diagnostic tests specific for the ILT gpI protein. Monoclonal antibodies are generated in mice utilizing this **virus** according to the PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials & Methods)

DETD S-SPV-017 is a swinepox **virus** that expresses at least two foreign genes. The gene for E. coli B-galactosidase (lacZ) and the gene for infectious bovine rhinotracheitis **virus** glycoprotein G (IBR gpG) were inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique. . .

DETD S-SPV-017 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 614-83.18 (see Materials and Methods) and **virus** S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING B-GALACTOSIDASE (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant **virus** designated S-SPV-017. This **virus** was assayed for B-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the **virus** was pure, stable, and expressing the foreign gene.

DETD . . . S-SPV-017 plaques and not with S-SPV-016 negative control plaques. All S-SPV-017 observed plaques reacted with the antiserum indicating that the **virus** was stably expressing the IBR

foreign gene. The assays described here were carried out in ESK-4 cells,
indicating that ESK-4. . .

DETD This **virus** is used as an expression vector for expressing IBR glycoprotein G (gpG). Such IBR gpG is used as an antigen to identify antibodies directed against the wild-type IBR **virus** as opposed to antibodies directed against gpG deleted IBR **viruses**. This **virus** is also used as an antigen for the production of IBR gpG specific monoclonal antibodies. Such antibodies are useful in the development of diagnostic tests specific for the IBR gpG protein. Monoclonal antibodies are generated in mice utilizing this **virus** according to the PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials & Methods).

DETD S-SPV-019 is a swinepox **virus** that expresses at least two foreign genes. The gene for E. coli .beta.-galactosidase (lacZ) and the gene for infectious bovine rhinotracheitis **virus** (IBRV) gE were inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction. . .

DETD S-SPV-019 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 708-78.9 (see Materials and Methods) and **virus** S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING .beta.-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant **virus** designated S-SPV-019. This **virus** was assayed for .beta.-galactosidase expression, purity and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue
indicating that the **virus** was pure, stable, and expressing the foreign gene.

DETD This **virus** is used as an expression vector for expressing IBR glycoprotein E (gpE). Such IBR gpE is used as an antigen to identify antibodies directed against the wild-type IBR **virus** as opposed to antibodies directed against gpE deleted IBR **viruses**. This **virus** is also used as an antigen for the production of IBR gpE specific monoclonal antibodies. Such antibodies are useful in the development of diagnostic tests specific for the IBR gpE protein. Monoclonal antibodies are generated in mice utilizing this **virus** according to the PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials & Methods).

DETD S-SPV-018 is a swinepox **virus** that expresses at least two foreign genes. The gene for E. coli B-galactosidase (lacZ) and the gene for pseudorabies **virus** glycoprotein E (PRV gpE) are inserted into the SPV 570-33.32 ORF (a unique PstI site has replaced the unique AccI. . .

DETD S-SPV-018 is derived from the S-SPV-001 (Kasza Strain). This is accomplished utilizing the final homology vector and **virus** S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock is screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING B-GALACTOSIDASE (BLUOGAL AND CPRG ASSAYS). Red plaque purification of the recombinant **virus** is designated S-SPV-018. This **virus** is assayed for B-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the **virus** is pure, stable, and expressing the foreign gene.

DETD This **virus** is used as an expression vector for expressing PRV

glycoprotein E (gpE). Such PRV gpE is used as an antigen to identify antibodies directed against the wild-type PRV **virus** as opposed to antibodies directed against gpE deleted PRV **viruses**. This **virus** is also used as an antigen for the production of PRV gpE specific monoclonal antibodies. Such antibodies are useful in the development of diagnostic tests specific for the PRV gpE protein. Monoclonal antibodies are generated in mice utilizing this **virus** according to the PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials & Methods).

DETD . . . site be in a region non-essential to the replication of the SPV

and that the site be flanked with swinepox **virus** DNA appropriate for mediating homologous recombination between **virus** and plasmid DNAs. The unique NdeI restriction site in plasmid 520-90.15 is located within the coding region of the SPV thymidine kinase gene (32). Therefore, we have shown that the thymidine kinase gene of swinepox **virus** is non-essential for DNA replication and is an appropriate insertion site.

DETD S-SPV-010 is a swinepox **virus** that expresses a foreign gene. The E. coli B-galactosidase (lacz) gene is inserted into a unique NdeI restriction site within. . . The foreign gene (lacZ) is under the control of the synthetic late promoter, LP1. We have shown that the swinepox **virus** thymidine kinase gene is non-essential for replication of the **virus** and is an appropriate insertion site.

DETD A 1739 base pair HindIII-BamHI fragment subcloned from the HindIII G fragment contains the swinepox **virus** thymidine kinase gene and is designated homology vector 520-90.15. The homology vector 520-90.15 was digested with Nde I, and AscI. . . was ligated into the Asc I site within the thymidine kinase gene. The recombinant homology vector 561-36.26 was cotransfected with **virus** S-SPV-001 by the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV and **virus** plaques expressing B-galactosidase were selected by SCREEN FOR RECOMBINANT SPV EXPRESSING B-GALACTOSIDASE (BLUOGAL AND CPRG ASSAY).

The final result of blue and red plaque purification was the recombinant

virus designated S-SPV-010. This **virus** was assayed for B-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques

observed were blue indicating that the **virus** was pure, stable and expressing the foreign gene. The assays described here were carried out in ESK-4 cells, indicating that. . .

DETD The development of vaccines utilizing the swinepox **virus** to express antigens from various disease causing microorganisms can be engineered.

DETD Transmissible Gastroenteritis **Virus**

DETD The major neutralizing antigen of the transmissible gastroenteritis **virus** (TGE), glycoprotein 195, for use in the swinepox **virus** vector has been cloned. The clone of the neutralizing antigen is disclosed in U.S. Ser. No. 078,519, filed Jul. 27,. . .

DETD We have cloned the major capsid protein of the porcine (swine) parvovirus (PPV) for use in the swinepox **virus** vector. The clone of the capsid protein is disclosed in U.S. Pat. No. 5,068,192 issued Nov. 26, 1991. It is. . .

DETD We have cloned the major neutralizing antigen of the swine rotavirus, glycoprotein 38, for use in the swinepox **virus** vector. The clone of glycoprotein 38 is disclosed in U.S. Pat. No. 5,068,192 issued Nov. 26, 1991. It is contemplated. . .

DETD Hog Cholera **Virus**

DETD The major neutralizing antigen of the bovine viral diarrhea (BVD) **virus** was cloned as disclosed in U.S. Ser. No. 225,032, filed Jul. 27, 1988. Since the BVD and hog cholera **viruses** are cross protective (31), the BVD **virus** antigen has been targeted for use in the swinepox **virus** vector. It is contemplated that the procedures that have been used to express PRV g50 (gpD) in SPV and are disclosed herein are applicable to BVD **virus**.

DETD A protective antigen of Serpulina hyodysenteriae (3), for use in the swinepox **virus** vector has been cloned. It is contemplated that the procedures that have been used to express PRV gp50 in SPV. . .

DETD Antigens from the following microorganisms may also be utilized to develop animal vaccines: swine **influenza virus**, foot and mouth disease **virus**, African swine fever **virus**, hog cholera **virus**, Mycoplasma hyopneumoniae, porcine reproductive and respiratory syndrome/swine infertility and respiratory syndrome (PRRS/SIRS).

DETD Antigens from the following microorganisms may also be utilized to develop animal vaccines: feline leukemia **virus**, feline immunodeficiency **virus**, feline herpesvirus, feline infectious peritonitis **virus**, canine herpesvirus, canine coronavirus, canine parvovirus, parasitic diseases in animals (including Dirofilaria immitis in dogs and cats), equine infectious anemia, Streptococcus equi, coccidia, emeria, chicken anemia **virus**, Borrelia bergdorferi, bovine coronavirus, pasteurella, haemolytica.

DETD Recombinant swinepox **viruses** express equine **influenza virus** type A/Alaska 91, equine **influenza virus** type A/Prague 56, equine herpesvirus type 1 gB, or equine herpesvirus type 1 gD genes. S-SPV-033 and S-SPV-034 are useful as vaccines against equine **influenza** infection, and S-SPV-038 and S-SPV-039 are useful as a vaccine against equine herpesvirus infection which causes equine rhinotracheitis and equine abortion. These equine **influenza** and equine herpesvirus antigens are key to raising a protective **immune response** in the animal. The recombinant **viruses** are useful alone or in combination as an effective vaccine. The swinepox **virus** is useful for cloning other subtypes of equine **influenza virus** (including EIVA/Miami/63 and EIVA/Kentucky/81) to protect against rapidly evolving variants in this disease. S-SPV-033, S-SPV-034, S-SPV-038, and S-SPV-039 are also useful as an expression vector for expressing equine **influenza** or equine herpesvirus antigens. Such equine **influenza** or equine herpesvirus antigens are useful to identify antibodies directed against the wild-type equine **influenza virus** or equine herpesvirus. The **viruses** are also useful to in producing antigens for the production of monospecific polyclonal or monoclonal antibodies. Such antibodies are useful. . . the development of diagnostic tests specific for the viral proteins. Monoclonal or polyclonal antibodies are generated in mice utilizing these **viruses** according to the PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials and Methods).

DETD S-SPV-033 is a recombinant swinepox **virus** that expresses at least two foreign genes. The gene for E. coli .beta.-galactosidase (lacZ) and the gene for equine **influenza virus** type A/Alaska 91 neuraminidase were inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique. . .

DETD S-SPV-033 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 732-18.4 (see Materials and Methods) and **virus** S-SPV-001 in the HOMOLOGOUS RECOMBINATION

PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING .beta.-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant **virus** designated S-SPV-033. This **virus** was assayed for .beta.-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue
indicating that the **virus** was pure, stable, and expressing the foreign gene.

DETD S-SPV-034 is a swinepox **virus** that expresses at least two foreign genes. The gene for E. coli .beta.-galactosidase (lacZ) and the gene for equine **influenza virus** type A/Prague 56 neuraminidase were inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique. . .

DETD S-SPV-034 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 723-59A9.22 (see Materials and Methods) and **virus** S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection
stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING .beta.-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant **virus** designated S-SPV-034. This **virus** was assayed for .beta.-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue
indicating that the **virus** was pure, stable, and expressing the foreign gene.

DETD . . . S-SPV-034 plaques and not with S-SPV-001 negative control plaques. All S-SPV-034 observed plaques reacted with the antiserum indicating that the **virus** was stably expressing the EIV PR/56 NA gene. The assays described here were carried out in ESK-4 cells, indicating that. . .

DETD S-SPV-038 is a swinepox **virus** that expresses at least two foreign genes. The gene for E. coli .beta.-galactosidase (lacZ) and the gene for equine herpesvirus. . .

DETD S-SPV-038 is derived from S-SPV-001 (Kasza Strain). This is accomplished
utilizing the homology vector 744-34 (see Materials and Methods) and **virus** S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock is screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING .beta.-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification is the recombinant **virus** designated S-SPV-038. This **virus** is assayed for .beta.-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the **virus** is pure, stable, and expressing the foreign gene.

DETD S-SPV-039 is a swinepox **virus** that expresses at least two foreign genes. The gene for E. coli .beta.-galactosidase (lacZ) and the gene for equine herpesvirus. . .

DETD S-SPV-039 is derived from S-SPV-001 (Kasza Strain). This is accomplished
utilizing the homology vector 744-38 (see Materials and Methods) and **virus** S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock is screened by the

SCREEN FOR RECOMBINANT SPV EXPRESSING .beta.-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification is the recombinant **virus** designated S-SPV-039. This **virus** is assayed for .beta.-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the **virus** is pure, stable, and expressing the foreign gene.

DETD Recombinant swinepox **viruses** express bovine respiratory syncytial **virus** attachment protein (BRSV G), BRSV Fusion protein (BRSV F), BRSV nucleocapsid protein (BRSV N), bovine viral diarrhea **virus** (BVDV) gp48, BVDV gp53, bovine **parainfluenza virus** type 3 (BPI-3) F, or BPI-3 HN. S-SPV-020, S-SPV-029, S-SPV-030, and S-SPV-032, S-SPV-028 are useful as vaccines against bovine disease. These BRSV, BVDV, and BPI-3 antigens are key to raising a protective **immune response** in the animal. The recombinant **viruses** are useful alone or in combination as an effective vaccine. The swinepox **virus** is useful for cloning other subtypes of BRSV, BVDV, and BPI-3 to protect against rapidly evolving variants in this disease.. . . Such BRSV, BVDV, and BPI-3 antigens are useful to identify antibodies directed against the wild-type BRSV, BVDV, and BPI-3. The **viruses** are also useful as antigens for the production of monospecific polyclonal or monoclonal antibodies. Such antibodies are useful in the development of diagnostic tests specific for the viral proteins. Monoclonal or polyclonal antibodies are generated in mice utilizing these **viruses** according to the PROCEDURE FOR PURIFICATION OF VIRAL GLYCOPROTEINS FOR USE AS DIAGNOSTICS (Materials and Methods).

DETD S-SPV-020 is a swinepox **virus** that expresses at least two foreign genes. The gene for E. coli .beta.-galactosidase (lacZ) and the gene for bovine respiratory syncytial **virus** (BRSV) G were inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction. . .

DETD S-SPV-020 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 727-20.5 (see Materials and Methods) and **virus** S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING .beta.-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant **virus** designated S-SPV-020. This **virus** was assayed for .beta.-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the **virus** was pure, stable, and expressing the foreign gene.

DETD . . . S-SPV-020 plaques and not with S-SPV-003 negative control plaques. All S-SPV-020 observed plaques reacted with the antiserum indicating that the **virus** was stably expressing the BRSV foreign gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4. . .

DETD S-SPV-029 is a swinepox **virus** that expresses at least two foreign genes. The gene for E. coli .beta.-galactosidase (lacZ) and the gene for bovine respiratory syncytial **virus** (BRSV) F were . inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction. . .

DETD S-SPV-029 was derived from S-SPV-001 (Kasza Strain). This was

accomplished utilizing the homology vector 727-20.10 (see Materials and Methods) and **virus** S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING .beta.-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant **virus** designated S-SPV-029.

DETD This **virus** was assayed for .beta.-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the **virus** was pure, stable, and expressing the foreign gene.

DETD . . . S-SPV-029 plaques and not with S-SPV-003 negative control plaques. All S-SPV-029 observed plaques reacted with the antiserum indicating that the **virus** was stably expressing the BRSV foreign gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4. . .

DETD S-SPV-030 is a swinepox **virus** that expresses at least two foreign genes. The gene for E. coli .beta.-galactosidase (lacZ) and the gene for bovine respiratory syncytial **virus** (BRSV) N were inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction. . .

DETD S-SPV-030 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 713-55.37 (see Materials and Methods) and **virus** S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING .beta.-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant **virus** designated S-SPV-030. This **virus** was assayed for .beta.-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the **virus** was pure, stable, and expressing the foreign gene.

DETD . . . S-SPV-030 plaques and not with S-SPV-003 negative control plaques. All S-SPV-030 observed plaques reacted with the antiserum indicating that the **virus** was stably expressing the BRSV foreign gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4. . .

DETD S-SPV-028 is a swinepox **virus** that expresses at least two foreign genes. The gene for E. coli .beta.-galactosidase (lacZ) and the gene for bovine **parainfluenza virus** type 3 (BPI-3) F were inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique. . .

DETD S-SPV-028 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 713-55.10 (see Materials and Methods) and **virus** S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING .beta.-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant **virus** designated S-SPV-028. This **virus** was assayed for .beta.-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue

indicating that the **virus** was pure, stable, and expressing the foreign gene.

DETD . . . S-SPV-028 plaques and not with S-SPV-003 negative control plaques. All S-SPV-028 observed plaques reacted with the antiserum indicating that the **virus** was stably expressing the BPI-3 foreign gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4. . .

DETD S-SPV-032 is a swinepox **virus** that expresses at least two foreign genes. The gene for E. coli .beta.-galactosidase (lacZ) and the gene for bovine viral diarrhea **virus** (BVDV) gp48 were inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction. . .

DETD S-SPV-032 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 727-78.1 (see Materials and Methods) and **virus** S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING .beta.-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant **virus** designated S-SPV-032. This **virus** was assayed for .beta.-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the **virus** was pure, stable, and expressing the foreign gene.

DETD S-SPV-040 is a swinepox **virus** that expresses at least two foreign genes. The gene for E. coli .beta.-galactosidase (lacZ) and the gene for bovine viral diarrhea **virus** (BVDV) gp53 were inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction. . .

DETD S-SPV-040 is derived from S-SPV-001 (Kasza Strain). This is accomplished utilizing the homology vector 738-96 (see Materials and Methods) and **virus** S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock is screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING .beta.-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification is the recombinant **virus** designated S-SPV-040. This **virus** is assayed for .beta.-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the **virus** is pure, stable, and expressing the foreign gene.

DETD . . . is manifested as the result of a combination of infectious diseases of cattle and additional stress related factors (52). Respiratory **virus** infections augmented by pathophysiological effects of stress, alter the susceptibility of cattle to Pasteurella organisms by a number of mechanisms.. . .

DETD The major infectious disease pathogens that contribute to BRD include but are not limited to infectious bovine rhinotracheitis **virus** (IBRV), **parainfluenza virus** type 3 (PI-3), bovine respiratory syncytial **virus** (BRSV), and Pasteurella haemolytica (53). Recombinant swinepox **virus** expressing protective antigens to organisms causing BRD is useful as a vaccine. S-SPV-020, S-SPV-029, S-SPV-030, S-SPV-032, and S-SPV-028 are useful.

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DETD Recombinant swinepox **viruses** S-SPV-031 and S-SPV-035 are useful as a vaccine against human disease. S-SPV-031 expresses the core antigen of **hepatitis** B **virus**. S-SPV-031 is useful against **hepatitis** B infection in humans. S-SPV-035 expresses the cytokine, interleukin-2, and is useful as an immune modulator to enhance an **immune response** in humans. When S-SPV-031 and S-SPV-035 are combined, a superior vaccine against **hepatitis** B is produced.

DETD S-SPV-031 is a swinepox **virus** that expresses at least two foreign genes. The gene for E. coli .beta.-galactosidase (lacZ) and the gene for **Hepatitis** B Core antigen were inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and the **Hepatitis** B Core antigen gene is under the control of the synthetic early/late promoter (EP1LP2).

DETD S-SPV-031 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 727-67.18 (see Materials and Methods) and **virus** S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING .beta.-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant **virus** designated S-SPV-031. This **virus** was assayed for .beta.-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the **virus** was pure, stable, and expressing the foreign gene.

DETD S-SPV-031 was assayed for expression of **Hepatitis** B Core antigen-specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Rabbit antisera to **Hepatitis** B Core antigen was shown to react specifically with S-SPV-031 plaques and not with S-SPV-001 negative control plaques. All S-SPV-031 observed plaques reacted with the antiserum indicating that the **virus** was stably expressing the **Hepatitis** B Core antigen gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4 cells would be. . .

DETD To confirm the expression of the **Hepatitis** B Core antigen gene product, cells were infected with SPV-031 and samples of infected cell lysates were subjected to SDS-polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. Rabbit antisera to **Hepatitis** B Core antigen was used to detect expression of **Hepatitis** B specific proteins. The lysate from S-SPV-031 infected cells exhibited a band at 21 kd which is the expected size of the **Hepatitis** B Core antigen.

DETD S-SPV-035 is a swinepox **virus** that expresses at least two foreign genes. The gene for E. coli .beta.-galactosidase (lacZ) and the gene for human IL-2. . .

DETD S-SPV-035 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 741-84.14 (see Materials and Methods) and **virus** S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING .beta.-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant **virus** designated S-SPV-035. This **virus** was assayed for .beta.-galactosidase

expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue
indicating that the **virus** was pure, stable, and expressing the foreign gene.

DETD Human vaccines using recombinant swinepox **virus** as a vector

DETD Recombinant swinepox **virus** is useful as a vaccine against human diseases. For example, human **influenza virus** is a rapidly evolving **virus** whose neutralizing viral epitopes rapidly change. A useful recombinant swinepox vaccine is one in which the **influenza virus** neutralizing epitopes are quickly adapted by recombinant DNA techniques to protect against new strains of **influenza virus**. Human **influenza virus hemagglutinin** (HN) and neuraminidase (NA) genes are cloned into the swinepox **virus** as described in CLONING OF EQUINE **INFLUENZA VIRUS HEMAGGLUTININ** AND NEURAMINIDASE GENES (See Materials and Methods and Example 17).

DETD Recombinant swinepox **virus** is useful as a vaccine against other human diseases when foreign antigens from the following diseases or disease organisms are expressed in the swinepox **virus** vector: **hepatitis** B **virus** surface and core antigens, **hepatitis** C **virus**, human immunodeficiency **virus**, human herpesviruses, **herpes** simplex **virus**-1, **herpes** simplex **virus**-2, human cytomegalovirus, Epstein-Barr **virus**, Varicella-Zoster **virus**, human herpesvirus-6, human herpesvirus-7, human **influenza**, **measles virus**, hantaan **virus**, pneumonia **virus**, rhinovirs, poliovirus, human respiratory syncytial **virus**, retrovirus, human T-cell leukemia **virus**, rabies **virus**, mumps **virus**, malaria (Pasmodium falciparum), Bordetelia pertussis, Diptheria, Rickettsia prowazekii, Borrlia bergdorferi, Tetanus toxoid, malignant tumor antigens.

DETD Furthermore, S-SPV-035 (Example 20), when combined with swinepox **virus** interleukin-2 is useful in enhancing **immune response** in humans. Additional cytokines, including but not limited to, interleukin-2, interleukin-6, interleukin-12, interferons, granulocyte-macrophage colony stimulating factors, interleukin receptors
from. . .

DETD Recombinant swinepox **virus** express foreign genes in a human cell line. We demonstrated that S-SPV-003 (EP1LP2 promoter expressing the lacZ gen) expressed the. . . gene in THP human monocyte cell lines by measuring .beta.-galactosidase activity. We did not observe any
cytopathic effect of swinepox **virus** on the THP human monocyte cells, indicating that recombinant swinepox **virus** can express foreign genes in a human cell line, but will not productively infect or replicated in the human cell line. We have demonstrated that swinepox **virus** replicates well in ESK-4 cells (embryonic swine kidney) indicating that ESK-4 cells would be a suitable substrate for the production. . .

DETD Avian vaccines using recombinant swinepox **virus** as a vector

DETD S-SPV-026 is a swinepox **virus** that expresses at least two foreign genes. The gene for E. coli .beta.-galactosidase (lacZ) and the gene for infectious bursal disease **virus** (IBDV) polyprotein were inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique AccI restriction. . .

DETD S-SPV-026 was derived from S-SPV-001 (Kasza Strain). This was

accomplished utilizing the homology vector 689-50.4 (see Materials and Methods) and **virus** S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING .beta.-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant **virus** designated S-SPV-026. This **virus** was assayed for .beta.-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue

indication that the **virus** was pure, stable, and expressing the foreign gene.

DETD . . . S-SPV-026 plaques and not with S-SPV-001 negative control plaques. All S-SPV-026 observed plaques reacted with the antiserum indicating that the **virus** was stably expressing the IBDV polyprotein gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4. . .

DETD S-SPV-027 is a swinepox **virus** that expresses at least two foreign genes. The gene for E. coli .beta.-galactosidase (lacZ) and the gene for infectious bursal disease **virus** (IBDV) VP2 (40 kd) were inserted into the SPV 617-48.1 ORF (a unique NotI restriction site has replaced a unique. . .

DETD S-SPV-027 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 689-50.7 (see Materials and Methods) and **virus** S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING .beta.-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant **virus** designated S-SPV-027. This **virus** was assayed for .beta.-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue

indicating that the **virus** was pure, stable, and expressing the foreign gene.

DETD . . . S-SPV-027 plaques and not with S-SPV-001 negative control plaques. All S-SPV-027 observed plaques reacted with the antiserum indicating that the **virus** was stably expressing the IBDV VP2 gene. The assays described here were carried out in ESK-4 cells, indicating that ESK-4. . .

DETD . . . are useful as vaccines against infectious bursal disease in chickens and also as expression vectors for IBDV proteins. Recombinant swinepox **virus** is useful as a vaccine against other avian disease when foreign antigens from the following diseases or disease organisms are expressed in the swinepox **virus** vector: Marek's disease **virus**, infectious laryngotracheitis **virus**, Newcastle disease **virus**, infectious bronchitis **virus** , and chicken anemia **virus**.

DETD S-SPV-036 is a swinepox **virus** that expresses at one foreign gene. The gene for E. coli .beta.-galactosidase (lacZ) was inserted into

the SPV 617-48.1 ORF. . .

DETD S-SPV-036 was derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing the homology vector 741-80.3 (see Materials and Methods) and **virus** S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING .beta.-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red

plaque purification was the recombinant **virus** designated S-SPV-036. This **virus** is assayed for .beta.-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the **virus** is pure, stable, and expressing the foreign gene.

DETD . . . promoter driving expression of a foreign gene in a poxvirus. S-SPV-036 is useful in formulating human vaccines, and recombinant swinepox **virus** is useful for the expression of neutralizing antigens from human pathogens. Recombinant swinepox **virus** expressed foreign genes in a human cell line as demonstrated by S-SPV-003 (EP1LP2) promoter expressing the lacZ gene) expressed .beta.-galactosidase. . .

DETD Recombinant swinepox **virus** expressed foreign genes in a human cell line as demonstrated by s-SPV-003 (EP1LP2 promoter expressing the lacz gene) expressed .beta.-galactosidase in THP human monocyte cell lines. THP human monocyte cells are useful for the production of recombinant swinepox **virus** as a human vaccine. Other cell lines in which swinepox **virus** will replicate include, but are not limited to, Vero cells (monkey), ST cells (swine testicle), PK-15 (porcine kidney), and ESK-4. . .

DETD Homology Vector 738-94.5 is a swinepox **virus** vector that expresses one foreign gene. The gene for E. coli .beta.-galactosidase (lacZ) was inserted into the the O1L open. . .

DETD . . . 5'-GCCTGAAGCTTCTAGTACAGTATTTACGACTTTTGAAAT-3' (SEQ ID NO. 188) to produce an 1123 base pair fragment with SalI and HindIII ends. A recombinant swinepox **virus** was derived utilizing homology vector 738-94.5 and S-SPV-001 (Kasza strain) in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The. . . SCREEN FOR RECOMBINANT SPV EXPRESSING .beta.-galactosidase (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification is the recombinant **virus**. This **virus** is assayed for .beta.-galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the **virus** is pure, stable, and expressing the foreign gene. Recombinant swinepox **viruses** derived from homology vector 738-94.5 are utilized as an expression vector to express foreign antigens and as a vaccine to raise a protective **immune response** in animals to foreign genes expressed by the recombinant swinepox **virus**. Other promoters in addition to the O1L promoter are inserted into the deleted region including LP1, EP1LP2, LP2EP2, HCMV immediate. . .

DETD 4. D. B. Boyle and B. E. H. Coupar, **Virus** Research 10, 343-356 (1988).

DETD . . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
- - (viii) POSITION. . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**

(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
- - (viii) POSITION. . . HYPOTHETICAL: YES
- - (iv) ANTI-SENSE: NO
- - (v) FRAGMENT TYPE: N-terminal
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: **Vaccinia** - #**virus**
(B) STRAIN: Copenhagen
- - (viii) POSITION IN GENOME:
(B) MAP POSITION: 23.2
(C) UNITS: %G
- - (xi) SEQUENCE. . . - - (iv) ANTI-SENSE: NO
- - (v) FRAGMENT TYPE: N-terminal
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
- - (viii) POSITION IN GENOME:
(B) MAP POSITION: 23.2
(C) UNITS: %G
- - (xi) SEQUENCE. . . HYPOTHETICAL: YES
- - (iv) ANTI-SENSE: NO
- - (v) FRAGMENT TYPE: C-terminal
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: **Vaccinia** - #**virus**
(B) STRAIN: Copenhagen
- - (viii) POSITION IN GENOME:
(B) MAP POSITION: 23.2
(C) UNITS: %G
- - (xi) SEQUENCE. . . - - (iv) ANTI-SENSE: NO
- - (v) FRAGMENT TYPE: C-terminal
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
- - (viii) POSITION IN GENOME:
(B) MAP POSITION: 23.2
(C) UNITS: %G
- - (xi) SEQUENCE. . .
DETD . . . - #, Robert J
Post, Leo - #nard E
(B) TITLE: DNA Sequence - # of the Gene for Pseudorabies
Virus gp5 - #0, a Glycoprotein without N-Linked
Glycosylatio - #n
(C) JOURNAL: J. Virol.
(D) VOLUME: 59
(E) ISSUE: 2
. . .
DETD Pseudorabies - # **Virus** Glycoproteins with
homology to
Herpes Si - #mplex **Virus** and
Varicella-Zoster **Virus**
Glycoprotein - #s
(C) JOURNAL: J. Virol.
(D) VOLUME: 60
(E) ISSUE: 1
(F) PAGES: 185-193
(G) DATE: Oct.-1986
-. . . - - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO

- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Newcastle - #disease **virus**
(B) STRAIN: B1
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 137-23.803 (- #PSY1142)
- - (viii) POSITION IN GENOME:
. . .
DETD . . . - - (iii) HYPOTHETICAL: N
- - (iv) ANTI-SENSE: N
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Pseudorabies - # **virus** \ Synthetic
oligonucleotid
primer
- - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:
- - CGCGAATTCG CTCGCAGCGC TATTGGC - # -. . . -
-
(iii) HYPOTHETICAL: N
- - (iv) ANTI-SENSE: N
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Pseudorabies - # **virus** \ Synthetic
oligonucleotid
e
primer
- - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:
- - GTAGGAGTGG CTGCTGAAG - # - #
. . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
- - (viii) POSITION. . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
- - (viii) POSITION. . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
- - (viii) POSITION. . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:

(B) CLONE: 515-85.1
- - (viii) POSITION. . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
- - (viii) POSITION. . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
- - (viii) POSITION. . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
- - (viii) POSITION. . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
- - (viii) POSITION. . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
- - (viii) POSITION. . .
DETD . . . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
- - (viii) POSITION. . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:

(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
- - (viii) POSITION. . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
- - (viii) POSITION. . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
- - (viii) POSITION. . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
- - (viii) POSITION. . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
- - (viii) POSITION. . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
- - (viii) POSITION. . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
- - (viii) POSITION. . . (genomic)

- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
- - (viii) POSITION. . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
- - (viii) POSITION. . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
- - (viii) POSITION. . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
- - (viii) POSITION. . .
DETD . . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
- - (viii) POSITION. . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
- - (viii) POSITION. . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza

(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
- - (viii) POSITION. . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
- - (viii) POSITION. . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
- - (viii) POSITION. . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
- - (viii) POSITION. . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
- - (viii) POSITION. . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
- - (viii) POSITION. . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
- - (viii) POSITION. . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO

- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
- - (viii) POSITION. . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
- - (viii) POSITION. . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
DETD . . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
- - (viii) POSITION. . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
- - (viii) POSITION. . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
- - (viii) POSITION. . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1

- - (viii) POSITION. . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
- - (viii) POSITION. . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
- - (viii) POSITION. . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
- - (viii) POSITION. . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
- - (viii) POSITION. . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
- - (viii) POSITION. . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
- - (viii) POSITION. . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza

(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
- - (viii) POSITION. . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
- - (viii) POSITION. . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
- - (viii) POSITION. . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
- - (viii) POSITION. . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
- - (viii) POSITION. . .
DETD . . . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
- - (viii) POSITION. . . (genomic)
- - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
- - (viii) POSITION. . . (genomic)
- - (iii) HYPOTHETICAL: NO

- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Swinepox - #**virus**
(B) STRAIN: Kasza
(C) INDIVIDUAL ISOLATE: - #S-SPV-001
- - (vii) IMMEDIATE SOURCE:
(B) CLONE: 515-85.1
- - (viii) POSITION. . . - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Infectious - #bovine rhinotracheitis **virus**
(B) STRAIN: Cooper Stra - #in
- - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:116:
- - CTGGTTCGGC CCAGAATTCG ATGCAACCCA CCGCGCCGCC. . . - (iii)
HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Infectious - #bovine rhinotracheitis **virus**
(B) STRAIN: Cooper Stra - #in
- - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:117:
- - CTCGCTCGCC CAGGATCCCT AGCGGAGGAT GGACTTGAGT. . .
DETD . . . (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Equine in - #fluenza A **hemagglutinin**
- - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:120:
- - GGAGGCCTTC ATGACAGACA ACCATTATTT TGATACTACT GA - #
- # 42
. . . (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Equine in - #fluenza A **hemagglutinin**
- - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:121:
- - GAAGGCCTTC TCAAATGCAA ATGTTGCATC TGATGTTGCC - #
- # 40
-. . . (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Equine In - #fluenza A **hemagglutinin**
(B) STRAIN: Prague/56
- - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:122:
- - GGGATCCATG AACACTCAAA TTCTAATATT AG - # -. . .
(iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Equine In - #fluenza A **hemagglutinin**
(B) STRAIN: Prague/56
- - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:123:
- - GGGATCCTTA TATACAAATA GTGCACCGCA - # - #
. . . - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Bovine pa - #rainfluenza-3 **virus**
- - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:130:
- - TTATGGATCC TGCTGCTGTG TTGAACAACT TTGT - # -
34
- -. . . - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Bovine pa - #rainfluenza-3 **virus**

- - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:131:
- - CCGCGGATCC CATGACCATC ACAACCATAA TCATAGCC - #
- # 38
-. . . - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Bovine pa - #rainfluenza-3 **virus**
- - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:132:
- - CGTCGGATCC CTTAGCTGCA GTTTTTTGGA ACTTCTGTTT TGA - #
- # 43
. . . - (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Bovine pa - #rainfluenza-3 **virus**
- - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:133:
- - CATAGGATCC CATGGAATAT TGGAAACACA CAAACAGCAC - #
- # 40
-. . . (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Bovine vi - #ral diarrhea **virus**
(B) STRAIN: Singer Stra - #in
- - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:134:
- - ACGTCGGATC CCTTACCAAA CCACGTCTTA CTCTTGTTTT. . . (iii) HYPOTHETICAL:
NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Bovine vi - #ral diarrhea **virus**
(B) STRAIN: Singer Stra - #in
- - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:135:
- - ACATAGGATC CCATGGGAGA AAACATAACA CAGTGGAACC. . . (iii) HYPOTHETICAL:
NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Bovine vi - #ral diarrhea **virus**
(B) STRAIN: Singer Stra - #in
- - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:136:
- - CGTGGATCCT CAATTACAAG AGGTATCGTC TAC. . . (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Bovine vi - #ral diarrhea **virus**
(B) STRAIN: Singer Stra - #in
- - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:137:
- - CATAGATCTT GTGGTGCTGT CCGACTTCGC A. . . (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Bovine re - #spiratory syncytial **virus**
(B) STRAIN: Strain 375
- - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:138:
- - TGCAGGATCC TCATTTACTA AAGGAAAGAT TGTTGAT - #
-. . . (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Bovine re - #spiratory syncytial **virus**
(B) STRAIN: Strain 375
- - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:139:
- - CTCTGGATCC TACAGCCATG AGGATGATCA TCAGC - #
-. . . (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:

(A) ORGANISM: Bovine re - #spiratory syncytial **virus**
(B) STRAIN: Strain 375
- - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:140:
- - CGTCGGATCC CTCACAGTTC CACATCATTG TCTTTGGGAT - #
. . . (iii) HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Bovine re - #spiratory syncytial **virus**
(B) STRAIN: Strain 375
- - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:141:
- - CTTAGGATCC CATGGCTCTT AGCAAGGTCA AACTAAATGA C -. . . (iii)
HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Bovine re - #spiratory syncytial **virus**
(B) STRAIN: Strain 375
- - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:142:
- - CGTTGGATCC CTAGATCTGT GTAGTTGATT GATTTGTGTG A -. . . (iii)
HYPOTHETICAL: NO
- - (iv) ANTI-SENSE: NO
- - (vi) ORIGINAL SOURCE:
(A) ORGANISM: Bovine re - #spiratory syncytial **virus**
(B) STRAIN: Strain 375
- - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:143:
- - CTCTGGATCC TCATACCCAT CATCTTAAAT TCAAGACATT A -. . .

CLM What is claimed is:

1. A recombinant swinepox **virus** which comprises a foreign DNA which (a) is inserted into a non-essential site located in the HindIII M fragment of. . . a polypeptide derived from a human pathogen and (b) is expressed in a host cell infected by the recombinant swinepox **virus**.

2. The recombinant swinepox **virus** of claim 1, wherein the polypeptide is derived from the group consisting of human herpesvirus, **herpes** simplex **virus**-1, **herpes** simplex **virus**-2, human cytomegalovirus, Epstein-Barr **virus**, Varicell-Zoster **virus**, human herpesvirus-6, human herpesvirus-7, human **influenza**, human immunodeficiency **virus**, rabies **virus**, **measles virus**, **hepatitis** B **virus** and **hepatitis** C **virus**.

3. The recombinant swinepox **virus** of claim 2, wherein the polypeptide is **hepatitis** B **virus** core protein or **hepatitis** B **virus** surface protein.

4. The recombinant swinepox **virus** of claim 3, designated S-SPV-031.

5. The recombinant swinepox **virus** of claim 1, wherein the polypeptide is associated with malaria.

6. The recombinant swinepox **virus** of claim 1, wherein the insertion site is present within the larger HindIII to BglII sub-fragment of the HindIII M. . .

7. The recombinant swinepox **virus** of claim 6, wherein the insertion site is within an open reading frame contained in the HindIII to BglII sub-fragment.

. . .

8. The recombinant swinepox **virus** of claim 7, wherein the insertion site is the AccI restriction endonuclease site located in the HindIII to BglII sub-fragment.

. . .

9. The recombinant swinepox **virus** of claim 8, wherein the AccI restriction endonuclease site is replaced by a NotI restriction endonuclease site.

10. The recombinant swinepox **virus** of claim 8, wherein the AccI restriction endonuclease site is replaced by a PstI restriction endonuclease site.

11. The recombinant swinepox **virus** of claim 1, further comprising an insertion site within an open reading frame encoding swinepox **virus** thymidine kinase.

12. The recombinant swinepox **virus** of claim 11, wherein the insertion site is the NdeI restriction endonuclease site located within the open reading frame encoding the swinepox **virus** thymidine kinase.

13. The recombinant swinepox **virus** of claim 12, wherein the NdeI restriction site is replaced by a AscI restriction endonuclease site.

14. The recombinant swinepox **virus** of claim 1, wherein the expression of the foreign DNA is under the control of a promoter located upstream from. . .

15. The recombinant swinepox **virus** of claim 14, wherein the promoter is an endogenous swinepox viral promoter or an exogenous promoter.

16. The recombinant swinepox **virus** of claim 15, wherein the exogenous promoter is a synthetic pox viral promoter.

17. The recombinant swinepox **virus** of claim 15, wherein the exogenous promoter is human cytomegalovirus immediately early gene promoter.

18. A recombinant swinepox **virus** which comprises a foreign DNA which (a) is inserted into a non-essential site located in the HindIII M fragment of the swinepox genome, wherein the foreign DNA encodes a cytokine capable of stimulating an **immune response** in a host infected by the recombinant swinepox **virus** and (b) is expressed in a host cell infected by the recombinant swinepox **virus**.

19. The recombinant swinepox **virus** of claim 18, wherein the cytokine is interleukin-2, interleukin-6, interleukin-12, an interferon, a granulocyte-macrophage colony stimulating factor, or an interleukin.

. .

20. The recombinant swinepox **virus** of claim 19, wherein the cytokine is human interleukin-2.

21. A recombinant swinepox **virus** which comprises a foreign DNA which (a) is inserted into a non-essential site of the swinepox genome, wherein the foreign. . . a polypeptide derived from an equine

pathogen and (b) is expressed in a host cell infected by the recombinant swinepox **virus**.

22. The recombinant swinepox **virus** of claim 21, wherein the polypeptide is derived from equine **influenza virus** or equine herpesvirus.

23. The recombinant swinepox **virus** of claim 22, wherein the polypeptide is equine **influenza virus** type A/Alaska 91 neuraminidase, equine **influenza virus** type A/Prague 56 neuraminidase, equine **influenza virus** type A/Miami 63 neuraminidase, equine **influenza virus** type A/Kentucky neuraminidase, equine herpesvirus type 1 glycoprotein B, or equine herpesvirus type 1 glycoprotein D.

24. The recombinant swinepox **virus** of claim 23, wherein the polypeptide is equine **influenza virus** type A/Alaska 91 neuraminidase.

25. The recombinant swinepox **virus** of claim 24, designated S-SPV-033.

26. The recombinant swinepox **virus** of claim 23, wherein the polypeptide is equine **influenza virus** type A/Prague 56 neuraminidase.

27. The recombinant swinepox **virus** of claim 26, designated S-SPV-034.

28. The recombinant swinepox **virus** of claim 23, wherein the polypeptide is equine herpesvirus type 1 glycoprotein B.

29. The recombinant swinepox **virus** of claim 28, designated S-SPV-038.

30. The recombinant swinepox **virus** of claim 23, wherein the polypeptide is equine herpesvirus type 1 glycoprotein D.

31. The recombinant swinepox **virus** of claim 30, designated S-SPV-039.

32. A recombinant swinepox **virus** which comprises a foreign DNA which (a) is inserted into a non-essential site located in the HindIII M fragment of the swinepox genome, wherein the foreign DNA encodes a polypeptide derived from bovine respiratory syncytial **virus** or bovine **parainfluenza virus**, and wherein the foreign DNA (b) is expressed in a host cell infected by the recombinant swinepox **virus**.

33. The recombinant swinepox **virus** of claim 32, wherein the polypeptide is bovine respiratory syncytial **virus** attachment protein (BRSV G), bovine respiratory syncytial **virus** fusion protein (BRSV F), bovine respiratory syncytial **virus** nucleocapsid protein (BRSV N), bovine **parainfluenza virus** type 3 fusion protein, or bovine **parainfluenza virus** type 3 **hemagglutinin** neuraminidase.

34. The recombinant swinepox **virus** of claim 33, wherein the polypeptide is bovine respiratory syncytial **virus** attachment protein (BRSV G).

35. The recombinant swinepox **virus** of claim 34, designated S-SPV-020.

36. The recombinant swinepox **virus** of claim 33, wherein the polypeptide is bovine respiratory syncytial **virus** fusion protein (BRSV F).

37. The recombinant swinepox **virus** of claim 36, designated S-SPV-029.

38. The recombinant swinepox **virus** of claim 33, wherein the polypeptide is bovine respiratory syncytial **virus** nucleocapsid protein (BRSV N).

39. The recombinant swinepox **virus** of claim 38, designated S-SPV-030.

40. The recombinant swinepox **virus** of claim 33, wherein the polypeptide is bovine **parainfluenza virus** type 3 fusion protein.

41. The recombinant swinepox **virus** of claim 40, designated S-SPV-028.

42. The recombinant swinepox **virus** of claim 32, wherein the polypeptide is bovine **parainfluenza virus** type 3 **hemagglutinin** neuraminidase.

43. A recombinant swinepox **virus** which comprises a foreign DNA which (a) is inserted into a non-essential site located in the HindIII M fragment of the swinepox genome wherein the foreign DNA encodes bovine viral diarrhea **virus** glycoprotein 48 or bovine viral diarrhea **virus** glycoprotein 53, and wherein the foreign DNA (b) is expressed in a host infected by the recombinant swinepox **virus** .

44. The recombinant swinepox **virus** of claim 43, designated S-SPV-032.

45. The recombinant swinepox **virus** of claim 43, designated S-SPV-040.

46. A recombinant swinepox **virus** which comprises a foreign DNA which (a) is inserted into a non-essential site located in the HindIII M fragment of the swinepox genome, wherein the foreign DNA encodes a polypeptide derived from infectious bursal disease **virus** and wherein the foreign DNA (b) is expressed in a host cell infected by the recombinant swinepox **virus**.

47. The recombinant swinepox **virus** of claim 46, wherein the polypeptide is infectious bursal disease **virus** polyprotein.

48. The recombinant swinepox **virus** of claim 46, wherein the

polypeptide is infectious bursal disease **virus** VP2.

L13 ANSWER 5 OF 285 USPATFULL
TI Retrovirus vectors derived from avian sarcoma leukosis **viruses** permitting transfer of genes into mammalian cells
PI US 6096534 20000801
WO 9637625 19961128
AB Recombinant avian sarcoma leukosis **virus** (ASLV)-derived retrovirus vectors having an expanded host range are described. The host range is expanded by the replacement of the ASLV envelope gene by an envelope gene from a **virus** capable of infecting both mammalian and avian cells. The resulting recombinant ASLV-derived retroviral vectors can replicate efficiently in avian cells,. . .
SUMM . . . of genetic engineering and gene transfer. More specifically, the invention relates to recombinant retrovirus vectors derived from avian sarcoma leukosis **viruses** (ASLVs) having an expanded host range. In particular, this invention relates to ASLV recombinant retrovirus vectors wherein a viral env gene derived from a **virus** capable of infecting both mammalian and avian cells is substituted for the ASLV env gene, allowing the vectors to efficiently. . .
SUMM . . . vectors carrying and expressing desired nucleic acid sequences in both cultured cells and intact animals. (Weiss et al, RNA Tumor **Viruses** (1982)).
SUMM Third, retroviral genomes are small, making it relatively easy to manipulate a cloned DNA copy of the genome. Moreover, the **viruses** are efficient; in culture, essentially all of the cells can be infected.
SUMM . . . rapid replication in these cells without aid of a helper or packaging cell line. This permits generation of high titer **virus** stocks by simply passaging transfected cells and allowing the **virus** to spread.
SUMM A number of retroviral vector systems have been described, including systems based on both mammalian (murine leukemia **virus**, Cepko, et al., (1984) Cell 37:1053-1062, Cone and Mulligan, (1984) PNAS (U.S.A.) 81:6349-6353; mouse mammary tumor **virus**, Salmons et al., (1984) Biochem. Biophys. Res. Commun. 159:1191-1198; gibbon ape leukemia **virus**, Miller et al. (1991) J. Virology, 65:2220-2224; human immunodeficiency **virus**, Buchschacher and Panganiban, (1992) J. Virology 66:2731-2739, Page et al., (1990) J. Virology 64:5270-5276) Shimada et al., (1991) J. Clin.. . .
SUMM For example, one of the most widely used retroviral vectors is a replication-defective derivative of Moloney murine leukemia **virus** (MLV). The main advantage of MLV is that it has a wide host range and can infect mammalian host cells, including human cells. However, the vectors derived from this **virus** are replication-defective. MLV vectors contain all of the cis-active elements necessary for viral replication, but lack the genes for the.
.
.
SUMM . . . helper or packaging cell line genome and the replication-defective vector can occur and can result in the generation of wild-type **virus**. (Ott et al., (1994) Hum. Gene Ther. 5:567-575). Contamination of the recombinant retroviral vector stock with replication-competent MLV can interfere. . .
SUMM Other frequently used retroviral vectors are derived from avian sarcoma leukosis **viruses** (ASLVs), particularly the Rous sarcoma **virus** (RSV). (Hughes and Kosik (1984) Virology 136:89-99; Hughes et al., (1987) J. Virology 61:3004-3012). RSV is the only known

replication-competent. . . from the RSV derived vector and replaced with a gene or genes of interest without affecting the ability of the **virus** to replicate. For example, retroviral vectors derived from RSV in which the v-src sequences present in the parental RSV have. .

.

J. Virology 65:3728-3737. (See also Hughes and Kosik (1984) Virology 136:89-99). Retrovirus vectors derived from replication competent endogenous Rous associated **virus** type-O (RAV-O) are designated RCOS (Greenhouse, et al., (1988) J. Virology 62:4809-4812). Vectors without splice acceptors are designated RCON and. . .

SUMM . . . the vector into cultured chicken embryo fibroblasts (CEFS) or other avian cells, and passaging the transfected cells and allowing the **virus** to spread. The simplicity of the **virus** stock preparation and the high titers that are easily achievable with the replication-competent retroviral vectors are significant advantages. Additionally, these. . .

SUMM . . . env glycoprotein. In RSV and other ASLVs, the env glycoprotein is restricted to binding to avian cell receptors. Thus, these **viruses** cannot infect mammalian cells efficiently.

SUMM . . . to overcome this limitation is to make transgenic mice that express the cellular receptor of subgroup A avian leukosis sarcoma **viruses**. (Federspiel et al., (1994) PNAS (U.S.A.) 91:11241-11245). RSV-derived vectors are able to transfer and stably express alkaline phosphatase and chloramphenicol-acetyltransferase. . . of the RSV-derived vectors is limited to the small number of mammalian host cells carrying the subgroup A avian leukosis **virus** receptor.

SUMM . . . be "packaged" by the envelope glycoproteins of other viral species. Through a mechanism that is not well understood, a pseudotyped **virus** bearing envelope glycoprotein that is a mixture of the two **viruses** is generated. (Emi et al. (1991) J. Virology 65:1201-1207). The pseudotyped **virus** has the host range of the **virus** donating the envelope protein. (Burns et al. (1993) PNAS (U.S.A.) 90:8033-8037).

SUMM . . . Emi et al. (1991) J. Virology 65:1201-1207 and Burns et al. (1993) PNAS (U.S.A.) 90:8033-8037 describe the generation of pseudotyped **viruses** by co-infection of the same cells with MLV and the vesicular stomatitis **virus** (VSV) helper/packaging **virus**. The resulting pseudotyped **viruses** have the increased host cell range of VSV (i.e. they can infect hamster cells, which MLV generally cannot infect) but. . .

SUMM Landau and Littman, (1992), J. Virology, 66:5110-5113, describe the production of replication-defective pseudotyped **viruses** wherein the MLV genome bears either the MLV or ecotropic or the RSV envelope glycoprotein. The packaging system is produced by transient expression of the env genes in cells infected with replication defective MLV. The resulting MLV pseudotyped **viruses** have expanded host ranges.

SUMM Researchers have attempted to "package" the ASLV genome in the envelope glycoprotein of a **virus** with a broader host range. For example, Weiss et al., (1977) Virology 77:808-825, describe superinfection of cells producing RSV with. . . sensitive mutants of VSV in an effort to expand the host range of the RSV-based vectors. Two types of pseudotyped **viruses** resulted: VSV genomes bearing RSV envelope antigens and RSV genomes bearing VSV envelope antigens. The RSV genomes bearing the VSV envelope antigens possessed the host range of the VSV **virus** and were capable of infecting mammalian cells,

but at a lower titer than chicken cells.

SUMM . . . cells was observed. However, the MLV env protein does not appear to compete efficiently with RSV env to form pseudotyped **virus**. In addition, the resulting RSV pseudotyped **viruses** with the xenotropic and ecotropic MLV env antigens were shown to infect mammalian cells only at a very low titer. . .

SUMM In each of these references the expanded host range avian pseudotyped **viruses** depend on the production of an envelope protein by a help **virus** or packaging cell. Thus, these vector systems are susceptible to recombination between the two viral genomes and the instability and potential contamination with wild-type **virus** recombination engenders. Consequently, they are not suitable for gene therapy.

SUMM Some researchers have attempted to expand the host cell range of avian leukosis **virus**-based vectors by creating recombinant vectors which express chimeric proteins with expanded host cell binding capacity. For example, Dong et al., (1992) J. Virology 66:7374-7382, describe a recombinant RSV-based vector expressing a chimeric **influenza virus hemagglutinin** (HA). Plasmids containing chimeric HA genes comprised of the coding sequence for the RSV env signal peptide fused to the **hemagglutinin** (HA) structural genes or a combination of HA and RSV structural genes were used to co-infect cells with plasmids carrying. . .

SUMM . . . infect a broad range of cell types in a variety of avian and mammalian species, independent of helper or packaging **virus** cell lines; and 2) unable to replicate once inside the mammalian host cell, until the present invention, such a vector. . .

SUMM The present invention, in general, provides recombinant avian sarcoma leukosis **virus** (ASLV)-derived retroviral vectors having an expanded host range, thereby allowing the vector to be used for gene transfer in a variety of species. In particular, the ASLV envelope gene is replaced by the env region derived from a **virus** capable of infecting both mammalian and avian cells. The recombinant retroviral vectors of this invention can replicate efficiently in avian. . .

DRWD . . . and 4 show RSVgagpol recovered from supernatants of cells transfected with RSVgagpol plasmid alone and Lanes 5 and 6 show **virus** particles recovered from supernatants of cells co-transfected with both plasmids.

DRWD . . . ability of RCAS-M(4070A) to replicate in CEFs, cells were transfected with RCAS-M(4070A) DNA and passaged six times to allow the **virus** to spread. At passages 1, 4 and 6 a small number of cells were plated separately. Those containing **virus** were identified by staining with antibodies that react with gp70. Only a small number of positively stained cells are seen. . .

DRWD . . . depiction of mutations in the amphotropic env gene of RCAS-M(4070A)NEO. CEFs were infected with RCAS-M(4070A)NEO after 3 passages of the **virus**. Full-length clones of the viral DNA were derived from the library of a low-molecular-weight DNA which was extracted from infected. . .

DRWD . . . by a RT assay. CEFs were transfected with plasmid DNA and passaged. 24 h after transfection and at each passage, **virus** particles were recovered from the culture fluid by centrifugation and quantified by determination of the RT activity. The control, uninfected. . .

DRWD . . . 9B: Titers of RCAS-M2(4070A)Puro1, 6, 8 and 9 on D17 cells. Cells were infected with the serial 10-fold dilutions of **virus** -containing culture fluid harvested at each passage. Resistant clones were selected in the medium containing puromycin. pac.sup.r colonies

were stained with. . .

DETD . . . functional equivalents thereof. More specifically, the recombinant retroviral vectors comprise LTR, pol and gag regions derived from avian sarcoma leukosis **viruses** (ASLV) and an env region derived from a **virus** capable of infecting both avian and mammalian cells, such as amphotropic MLV. The recombinant vectors are capable of carrying and. . .

DETD . . . Examples of the genomes from which the gag sequence may be derived include, but are not limited to, Rous sarcoma **virus** (RSV), MC29-associated **virus** (MAV), Rous associated **virus** (RAV), RAV-O, avian erythroblastosis **virus** (AEV), avian myoblastosis **virus** (AMV), other members of this **virus** family and their associated helper **viruses**. The gag region may comprise all or parts of the gag gene. For a replication competent vector, it is preferable. . .

DETD . . . sequences may be derived, include, but are not limited to, RSV, MAV, RAV, RAV-O, AEV, AMV, other members of this **virus** family and their associated helper **viruses**. The pol region may comprise all or parts of the pol gene. For a replication competent vector it is preferable. . .

DETD Env is the retroviral gene which encodes the envelope antigens that determine the antigenic and subgroup specificity of the **virus**. The env sequence is preferably derived from the envelope region of a **virus** capable of infecting both mammalian and avian cells. Examples of the genomes from which the env sequences may be derived include, but are not limited to, mammalian **viruses** capable of infecting avian species, such as the amphotropic Moloney murine leukemia **virus** (MLV) env sequence. Weiss et al., (1982) RNA Tumor **Viruses**; Weiss (1985) Supplement to RNA Tumor **Viruses**. The env sequence may comprise all or parts of the env gene. For a replication competent **virus**, it is preferable that a sequence sufficient to encode a functional envelope protein is used. By way of example, a. . .

DETD In a preferred embodiment, the coding sequence of a **virus** capable of infecting both mammalian and avian cells such as amphotropic MLV, is "adapted" to permit initial replication rates similar. . . 242 with isoleucine. As described more fully in the examples, the "adapted" env sequence may be produced by passaging the **virus** and selecting for fast replicating clones. Additionally, one skilled in the art will recognize that the env sequence may be. . .

DETD . . . retroviral vector of this invention, comprising the coding region of a wild type or "adapted" env gene sequence of a **virus** capable of infecting mammalian and avian cells and the N terminal signal peptide sequence of an ASLV.

DETD . . . are not limited to, RSV, MAV, RAV, RAV-O, AEV, AMV and other members of this family, and their associated helper **virus**. (Hughes and Kosik, (1984) Virology 136:89-99, Hughes et al., (1987) J. Virology 61:3005-3102). For a replication competent **virus**, preferably the entire LTR is used. Alternatively, in another embodiment, a retroviral vector with a single LTR may be used.. . .

DETD . . . another embodiment of this invention, the envelope region of the recombinant retroviral vectors of the invention is derived from a **virus** that recognizes mammalian, but not avian cell receptors. In order for a vector containing such an envelope sequence to infect.

.

.
DETD . . . replicate in avian cells it is important not to delete any of the gag, pol, or env sequences necessary for **virus** replication. Any restriction endonuclease site may be generated, preferably an endonuclease unique to the retroviral expression vector or an endonuclease. . .

DETD . . . plasmid may be introduced into the avian cells by conventional methodology and the cells may be passaged to allow the **virus** to spread throughout the culture. Subsequent generations of the **virus** may be used to infect other cells. Alternatively, a **virus** carrying the recombinant retroviral vector may be used to infect the avian cells. The viral stock produced may be used. . .

DETD . . . need of such therapy in a variety of ways. Retroviral supernatants from avian host cells transfected with and producing the **virus** may be administered to the individual in need of gene therapy. Additionally, a substantially purified form of the **virus** may be administered to the mammal in need of such treatment alone or in the form of a pharmaceutical composition.

DETD . . . in one embodiment, which takes advantage of the ability of the retroviral vectors of the invention to replicate and produce **virus** in avian but not mammalian cells, avian host cells containing the retroviral vector are administered to a mammal in need. . .

DETD . . . intralesional, subcutaneous or intraperitoneal injection or implantation. Alternatively, the cells containing the recombinant retroviral vectors may be administered locally by **topical** application, direct injection into an affected area or implantation of a porous device containing cells from the host or another. . .

DETD . . . or shortly after the onset of the disease of interest (or symptoms of the disease of interest) to enhance the **immune response** of a patient (a mammal, preferably a human) to the disease of interest and to attenuate the disease.

DETD . . . retroviral vectors may be directly administered to the mammal several ways, including, but not limited to exposure of cells to **virus** ex vivo or injection of the retrovirus into the affected tissue or intravenously. Alternatively, viral particles carrying all or part. . . of the nucleic acid sequences of interest may be administered locally by direct injection into an affected area or by **topical** application in a pharmaceutically acceptable carrier.

DETD . . . know the parameters to determine the correct titer of particles to be administered. The quantity of recombinant retroviral vector or **virus** carrying all or part of the nucleic acid sequence encoding the immunogenic protein of interest to be administered may be based on the titer of **virus** particles. The amount of **virus** to be administered is in no way limited to a particular concentration and may vary depending upon the individual being healed. Based on clinical parameters the treating physician will determine the therapeutically effective amount of the **virus** containing the gene of interest to be administered to a given individual. Such therapy may be administered as often as. . .

DETD Transfection of Cells and Preparation of **Virus** Particles

DETD . . . saline (PBS) and incubated in a growth medium for 24 hr. When necessary, transfected cells were passaged to allow the **virus** to spread through the culture.

DETD **Virus**-containing culture fluid was then harvested. 24 hr after transfection, the culture medium was harvested and viral particles were recovered by ultracentrifugation. To prepare **virus** particles,

culture fluid was clarified by low-speed centrifugation and the **virus** was pelleted through 15% sucrose cushion in SW41 rotor (Beckman) at 35,000 rpm for 1 hr at +4.degree. C. The. . .

DETD . . . medium (FIG. 2B, lanes 1 and 2). Transfection of RSVgagpol results in a synthesis of capsid proteins that assemble into **virus**-like particles that can be recovered by ultracentrifugation (FIG. 2B, lanes 3 and 4). More than one band was detected by. . .

DETD . . . MLV envelope protein assembles with RSV gag, recombinant RSV genome was constructed in which the envelope gene in the parental **virus** was replaced with the env gene of an amphotropic MLV. To ensure the efficient intracellular transport of the envelope precursor. . .

DETD . . . CEFs was measured. CEF cells were transfected with RCAS-M(4070A) DNA as described in Example 1 and passaged to allow the **virus** to spread. A total of six passages were done. At passages 1, 4 and 6, a small number of cells was plated separately, and the ability of the **virus** to spread was measured by the staining of CEFs by the antibodies that react with expressed gp70.

DETD To monitor the production of **virus** particles, cell culture medium at each passage was harvested and particles were recovered by ultracentrifugation as described in Example 1.. . .

DETD CEFs were transfected with RCAS-MC(4070A)Puro as described in Example 1 and passaged 6 times. **Virus**-containing culture fluid was used to infect fresh cells that were passaged two more times. The resulting **virus** was titered on murine cells (NIH 3T3), human (HeLa) cells and D17, dog kidney cells. Titration on mammalian cells was performed as follows. **Virus**-containing culture fluid was harvested and filtered through a 0.45 .mu.m membrane. Host cells were plated in 60-mm plates (5.times.10.sup.5 cells. . .

DETD . . . were generated 24 hours after transfection were titered on murine NIH 3T3 and human HeLa cells as described above. The **virus** obtained by the transient expression of the RCAS-M(4070A)NEO in CEFs had the titer 2-3.times.10.sup.3 colony forming units (cfu/ml) on both. . .

DETD The transfected CEFs were passaged and the produced **virus** was used to infect both fresh CEFs and mammalian cells. By immunoblotting analysis of the viral particles collected at each passage, it was determined that the **virus** replicated reasonably efficiently in CEFs; however, the ability to transfer neo.sup.r into NIH 3T3 cells decreased relatively rapidly (Table 2). . .

DETD TABLE 2

Titer of RCAS-M(4070A)NEO on NIH 3T3 cells

Virus	Titer, cfu/ml
24 h posttransfection	2.6 .times. 10.sup.3
Virus passage 1	10.sup.1
Virus passage 2	10.sup.1
Virus passage 3	5 .times. 10.sup.2

DETD In the murine leukemia **viruses**, the env precursor is initially cleaved by a cellular protease into gp70 (SU) and pre-p15E (TM). After the **virus** particle is released from the cell, the viral protease removes the C-terminal 16 residues from the cytoplasmic domain of pre-p15E,. . .

DETD Virions produced by cells infected with RCAS-M(4070A) do not contain MLV

protease. However, the chimeric **virus** is infectious, suggesting that pre-p15E is cleaved. Viral particles were prepared as described in Example 1 and the proteins were. . . by immunoblotting with rabbit antiserum against p15E (kind gift of Alan Rein) (FIG. 7). The data shows that the chimeric **virus** particles formed by RCAS-M(4070A) contain the processed p15E, although the MLV protease is not present. The transmembrane protein processing in. . .

DETD **Virus** Particles are not Produced by Mammalian Cells Infected with RCAS-M(4070A)

DETD . . . cells (Vogt et al., (1982) J. Virology 44:725-730) were implied as the possible defects that prevent the synthesis of the **virus** structural proteins and production of the **virus** particles.

DETD To determine whether the mammalian cells infected by RCAS-M(4070A) generate **virus** particles, we infected fresh NIH 3T3 cells with the supernatants of several NIH 3T3/RCAS-M(4070A)NEO clones carrying un-rearranged proviruses. Cells were. . .

DETD Initially the chimeric **virus** RCAS-M(4070A) replicates at a considerably lower rate than the parental vector RCASBP(A). Indeed, after transfection of RCAS-M(4070A) into CEFs, 3-4 cell passages were required before detectable amounts of the **virus** were produced. However, after 5-6 passages on CEFs, the chimeric **virus** infects these cells efficiently and spreads quickly throughout the entire culture.

DETD . . . investigate the possibility that genetic changes in the viral genome during this period of initial slow replication permitted the chimeric **virus** to adapt and grow more efficiently, the env regions of molecular clones of RCAS-M(4070A) provirus containing the neo marker gene. . .

DETD . . . be critically important for efficient replication, since it is present in all of the clones we derived from passaged RCAS-M(4070A)NEO **virus**. Furthermore, this mutation alone is sufficient to dramatically increase the efficiency of the **virus** replication, since it is the only mutation found in the env gene of clone env8, the **virus** that replicates most efficiently in CEFs and produces the highest titers on mammalian cells.

DETD . . . in gp70 increases the affinity of the protein for the avian receptor, it does not compromise the ability of the **virus** to infect mammalian cells.

DETD The resulting RCAS-M2(4070A)Puro1, 6, 8, and 9 vectors were transfected into CEFs, and the cells were passaged to generate **virus**. At each passage, the production of viral particles was quantified by determining the virion-associated RT activity, and the number of **viruses** able to infect mammalian cells was determined by titration on D17 cells as described in Example 2.

DETD Determination of the virion-associated RT activity was performed as described in Whitcomb et al., (1995) J. Virol 69:6228-6238. Briefly, **virus** particles were recovered from the culture fluid by centrifugation at 14,000 rpm for 30 min at 4.degree. C. Pellets were. .

DETD . . . in FIG. 9A, the initial rates of replication of the "adapted" vectors are much faster than the parental vector, RCAS-M(4070A)Puro. **Virus** production is detectable at passage 1 and reaches a maximum at passages 3-4, which is similar to the initial rates. . . The "adapted" RCAS-M2(4070A)Puro vectors are infectious for mammalian cells, exhibiting significant titers on D17 cells (FIG. 9B). The fastest replicating **virus** (clone env8) reaches the maximum titer on

D17 cells by passage 2 (compare FIGS. 9A and B).

DETD . . . was transfected into CEFs as described in Example 1 and the cells were passaged. At each passage, the number of **virus** particles able to infect mammalian cells was quantified by titration on D17 cells as described in Example 2. As can be seen in FIG. 9C, the RCAS-M2C(4070A)Puro replicates efficiently in CEFs, and the titer of a **virus** stock produced at each passage is the same as the titer of the RCAS-M2(4070A)Puro **virus** from which it was derived.

DETD . . . al., (1995) PNAS (USA) in press, Vogt et al., (1988) J. Virol 44:725-730) appear to prevent the production of infectious **virus** particles. To determine whether the mammalian cells infected by RCAS-M(4070A)NEO generate infectious **virus** particles, we infected fresh NIH 3T3 cells with the supernatants of several NIH 3T3/RCAS-M(4070A)NEO clones carrying unrearranged proviruses as described. . . G418 selection as described in Example 4. Similar experiments were performed using RCAS-M2(4070A)Puro to infect the mammalian cells and no **virus** particles were detected in the infected murine NIH 3T3 cells. Since it is inherently defective in the mammalian cells, the. . .

CLM What is claimed is:

. . . An isolated nucleic acid molecule comprising: a. at least one long terminal repeat (LTR) isolated from an avian sarcoma leukosis **virus** (ASLV): b. a first nucleic acid sequence selected from a gag region isolated from ASLV: c. a second nucleic acid. . .

. . . nucleic acid molecule of claim 1 wherein the LTR, the gag and the pol

sequences are isolated from Rous sarcoma **virus** (RSV).

5. The nucleic acid molecule of claim 1 wherein the envelope sequence is

isolated from the amphotropic murine leukemia **virus** (MLV).

L13 ANSWER 6 OF 285 USPATFULL

TI Method of inducing an **immune response** using **vaccinia virus** recombinants encoding GM-CSF

PI US 6093700 20000725
WO 9531105 19951123

AB A method of inducing expression of immune active cytokines in tumors in situ is provided wherein a **vaccinia virus** vector capable of inducing expression of a selected cytokine is generated and injected into a tumor so that cells of the tumor express the selected cytokine. A method of enhancing immunity in a host by administration of a **vaccinia virus** vector is also provided. Methods of treating cancer by administration of these **vaccinia virus** vectors are also provided.

SUMM . . . Bacillus Calmette-Guerin (BCG). The most effective of these approaches has been localized therapy with BCG for melanoma metastasis to the **skin** and superficial bladder cancer. While the mechanism of action of BCG is not completely understood, studies clearly

show that successful. . .

SUMM Cytokines such as the interleukins are important mediators in cell-mediated **immune responses** in a host. The cell-mediated **immune response** ("local **immune responses**") is produced by thymus derived lymphocytes or T-cells. T-cells detect the presence of invading pathogens through a recognition system referred. . . variety of regulatory and defense functions and play a central role in immunologic responses. When stimulated to produce a cell-mediated **immune response**

, some T-cells respond by acting as killer cells, killing the host's own cells when these have become infected with **virus** and possibly when they become cancerous and therefore foreign. Some T-cells respond by stimulating B cells while other T-cells respond by suppressing **immune responses**.

SUMM Examples of other interleukins which are mediators in cell-mediated **immune responses** include interferon-.gamma. (IFN-.gamma.), granulocyte-macrophage colony stimulating factor (GM-CSF), interleukin-4 (IL-4), interleukin-5 (IL-5) and interleukin-12 (IL-12). IFN-.gamma. activates macrophages and enhances. . .

SUMM . . . now been found that expression of immune active cytokines in tumors can be induced in situ by administration of a **vaccinia virus** vector. These **vaccinia virus** vectors can be administered to animals suffering from cancer as a treatment. The **vaccinia virus** vectors of the present invention are also useful in enhancing immunity to parasites and other invading pathogens which alone fail to invoke an effective host **immune response**.

SUMM . . . is to provide a method of inducing expression of immune active cytokines in tumors in situ which comprises generating a **vaccinia virus** vector capable of inducing expression of a selected cytokine and injecting the **vaccinia virus** vector into a tumor so that cells of the tumor express the selected cytokine.

SUMM . . . object of the present invention is to provide a method of enhancing immunity in a host which comprises generating a **vaccinia virus** vector capable of inducing expression of a selected cytokine and injecting the **vaccinia virus** vector into a host so that cells of the host express the selected cytokine.

SUMM . . . to provide a method of treating cancer which comprises administering to an animal suffering from cancer an amount of a **vaccinia virus** vector capable of inducing an **immune response** to the cancer in the animal.

DRWD FIG. 1 is a bar graph showing systemic immunity resulting from intravesical instillation of the **vaccinia virus** vector (VAC). Mice received intravesical instillation of VAC at 10, 100 and 1,000 plaque forming units (pfu). Two weeks later,. . .

DETD . . . invention, a method of inducing expression of immune active cytokines in tumors in situ is provided which comprises generating a **vaccinia virus** vector capable of inducing expression of a selected cytokine and injecting the **vaccinia virus** vector into a tumor so that cells of the tumor produce the selected cytokine. By the term "inducing" or "induces". . . measurable by methods well known in the art and that the level of expression of the cytokine results in an **immune response**. By the term "immune active cytokine" or "selected cytokine" it is meant to refer to any cytokine associated with an **immune response** leading to tumor destruction. Examples of such cytokines include, but are not limited to, interferon-.gamma. (IFN-.gamma.), granulocyte-macrophage colony stimulating factor (GM-CSF), interleukin-2 (IL-2), interleukin-4(IL-4), interleukin-5 (IL-5), and interleukin-12 (IL-12). The **vaccinia virus** vector may further comprise a gene for an immune accessory molecule such as B7.1 or B7.2. By "immune accessory molecule". . . active cytokine can make the tumor more immunogenic. Unlike in vitro methods of gene transfer, infection and transfection using recombinant **vaccinia**

has been found to be a simple, rapid and highly efficient procedure. **Vaccinia** recombinants can efficiently deliver antigens to the class I presentation pathway and have been proposed as feasible vectors for expressing protective antigens for vaccine delivery. Moss B and Flexner C., "**Vaccinia virus** expression vector", Ann. Rev. Immunol. 1987 5:305-324. The potential utility of **vaccinia** recombinants for intravesical gene therapy aimed at enhancing the immunogenicity of bladder tumor cells was suggested by Lee SS, et. .

.

DETD **Vaccinia virus**, a double stranded DNA poxvirus, has been well characterized since its successful use as a live vaccine to prevent smallpox. As a versatile eukaryotic expression vector, **vaccinia virus** can be genetically constructed to contain large fragments of foreign DNA (up to 25 kd) which have no effect on viral replication. Immunization with recombinant **vaccinia** can induce protective responses to the foreign gene(s) expressed. In the present invention a **vaccinia virus** vector (VAC) capable of inducing expression of a selected gene is generated in accordance with methods well known in the art. The **vaccinia virus** vector may further comprise genes encoding immune accessory molecules which in conjunction with the immune

active cytokine can make the. . .

DETD . . . other cells. The ability to express an antisense oligonucleotide complementary to the IL-10 DNA or mRNA can be incorporated into **vaccinia virus** vectors of the present invention to inhibit IL-10 production in tumor cells, thus enhancing the immunogenicity of these tumors.

DETD . . . insertion of the selected gene in the plasmid is confirmed by exploiting the high transfectability of certain cell lines following **vaccinia** infection. After 30 minutes of exposure to wild-type **vaccinia** at a multiplicity of 10:1, mouse L929 cells are transfected with a plasmid DNA-lipofectin (Gibco/BRL, Bethesda, Md.) mixture. Within hours. . . the up- and downstream halves of the VAC thymidine kinase gene. Following infection of CV-1 monkey kidney cells with non-recombinant **virus**, the plasmid is delivered using calcium phosphate precipitation. In a portion of the cells, the plasmid recombines into the **vaccinia** genome, disrupting the thymidine kinase gene. The resulting recombinants are then selected from wild-type

by growth in thymidine kinase negative 143B human osteosarcoma cells in the presence of bromodeoxyuridine. It is preferred that the Wyeth strain

of **vaccinia**, available from the Centers for Disease Control in Atlanta, Ga. (CDC) be used as this strain was used for small pox vaccinations in the United States. However, attenuated strains of **vaccinia** may also be used if immunogenicity following attenuation is not significantly compromised.

DETD Susceptibility of cells to the **vaccinia virus** was demonstrated in in vitro experiments in both murine and human tumor cells. Both type of cells were infected/transfected by **vaccinia** recombinants. Significant infection/transfection of established tumors in mice was also observed following intravesical administration. Systemic immunity to **vaccinia** did not inhibit tumor transfection by intravesically instilled **vaccinia** recombinants.

DETD . . . safety and maintained function of the viral gene over repeated administrations have also been demonstrated in humans. Five patients with **dermal**, subcutaneous and/or lymph node metastases from cutaneous melanoma were vaccinated with wild-type **vaccinia**

virus and, four days later, began intratumoral injections of the same vaccine. Escalating doses of up to 10.sup.7 pfu were safely administered repeatedly with only local and mild systemic reactions. Four of the patients developed anti-**vaccinia virus** antibody titers .gtoreq.1/3200. With rising antibody titers, local and systemic reactions decreased. One patient with a large exophytic lesion experienced dramatic tumor regression with multiple injections of 10.sup.7 pfu of **virus**. Sequential biopsies of this lesion over a two month period demonstrated repeated infection over successful production of viral gene protein. . .

DETD The **vaccinia virus** vectors of the present invention can also be used to enhance immunity in a host. In the present invention methods of enhancing immunity in a host are provided which comprise generating a **vaccinia virus** vector capable of inducing expression of a selected cytokine and injecting the **vaccinia virus** vector into a host so that cells of the host express the selected cytokine. By "host" it is meant to. . . to, mammals, fish, amphibians, reptiles, birds, marsupials, and most preferably, humans. This method is also useful in enhancing a host's **immune response** to parasites and other invading pathogens which alone may not invoke an **immune response**.

DETD In addition, the **vaccinia virus** vectors of the present invention can be used to mediate cytokine gene transfer into tumors with resultant production of soluble product. For example, a recombinant **vaccinia virus** containing the murine GM-CSF gene under the control of the early/late P7.5 **vaccinia** promoter (VV-GM) was constructed. VV-GM infected murine melanoma (B16.F10) and bladder (MB49) tumors were shown to produce high levels of. . .

DETD . . . of cancer. Methods of treating cancer are provided comprising administering to an animal suffering from cancer an amount of a **vaccinia virus** vector capable of inducing an **immune response** to the cancer in the animal. In a preferred embodiment, the **vaccinia virus** vector used comprises at least one gene for expression of a cytokine, preferably the gene for IFN-.gamma., GM-CSF, IL-4, IL-5 or IL-12. In this treatment, the **vaccinia virus** vector is placed in contact with the tumor in situ either by intravesical administration or by direct injection into the. . .

DETD The susceptibility of human prostatic carcinoma cells to **vaccinia** was also examined utilizing a recombinant vector encoding the human **influenza hemagglutinin** antigen HA. In vitro exposure of the prostatic cell lines LNCAP and PC3 to the **virus** followed by immunohistochemical staining of the encoded HA protein demonstrated a high efficiency in tumor infection/transfection. Thus, the **vaccinia virus** vectors of the present invention can also be used in the localized therapy of prostate cancer.

DETD The **vaccinia virus** vectors of the present invention are administered in a vaccine formulation comprising an effective concentration of **vaccinia virus** vector and a pharmaceutically acceptable carrier. By "effective concentration" it is meant an amount of **vaccinia virus** vector which when administered to a tumor results in measurable expression of the selected cytokine and an enhanced **immune response**. Such amounts can be routinely determined by one of skill in the art in

accordance with this disclosure. Pharmaceutically acceptable. . . substances which are added to therapeutic or prophylactic agents, for example vaccines or antigens used for immunization, to stimulate the **immune response**. Use of adjuvants in vaccines to enhance an **immune response** is well known in the art.

DETD Recombinant **Vaccinia Virus**

DETD Recombinant **vaccinia viruses** H1-VAC and NP-VAC expressing the **hemagglutinin** (H1) and nucleoprotein (NP) genes derived from **influenza virus** A/PR8/34 were used. Expression of both **influenza** polypeptides is under the control of the early/late 7.5 K promoter. Viral stocks quantitated in pfu were maintained in BSS/BSA. . .

DETD Supernatants from hybridoma cell lines specific for the **influenza** A **hemagglutinin** (H28-E23) and nucleoprotein antigens (HB65) were used to stain cells and tissues. The **virus** infected bladder tumor cells and bladder urothelium sections were fixed with cold acetone and blocked with 0.1% fetal calf serum.. . .

DETD . . . NP-VAC (10 pfu/cell) in BSS/BSA by incubating at 37.degree. C., 9% CO.sub.2 for 1 hour with rocking every 15 minutes. **Virus** was aspirated, media was added and the plate were incubated for another 4 hours. The cells were fixed with 1:1 acetone:methanol for 1 minute and washed with PBS before immunohistochemical staining. Uninfected and recombinant **virus** infected L929 fibroblasts, which are known to be susceptible to **vaccinia virus** infection, were used as a negative and positive control, respectively.

DETD . . . immunohistochemical staining with specific antibodies showed positive expression for encoded HA or NP antigens indicated by the cytoplasmic staining of **virus** infected TCC cells. In addition, the human bladder rumor cell line T24 and a human melanoma line were similarly infected. . .

DETD In vivo Assessment of **Virus** Infection and Transfection

DETD . . . 4-6 weeks of age, were purchased from the Jackson Laboratory, Bar Harbor, Me. The mice were intravesically instilled with recombinant **vaccinia virus**. Mice were anesthetized, catheterized via the urethra, then cauterized with a cautery wire (Birtcher Hyfricator, El Monte, Calif.) by applying. . . instilled with 10.sup.4 MB49 cells to establish intravesical growth of a tumor or either 10, 100 or 1,000 pfu of **vaccinia virus** recombinants in PBS. At 8 and 22 hours following instillation, mice were sacrificed and bladders were removed and frozen in. . .

DETD Mice, pre-immunized intraperitoneally with wild-type WR **vaccinia** (10.sup.7 pfu), were implanted intravesically with MB-49 tumor cells. Two weeks following tumor development, a single intravesical instillation of NP-VAC. . .

DETD Cytotoxic T Lymphocyte (CTL) responses to intravesical infection by **vaccinia** recombinants were determined by a 4 hour .sup.51 Cr assay. Spleens of **virus** infected mice were isolated at 2 weeks post-intravesical instillation, restimulated in vitro with live **virus** infected syngeneic spleen stimulators (3:1) and cultured for 7 days at 37.degree. C., 5% CO.sub.2. The responder cells were assayed for cytotoxicity on .sup.51 Cr labeled **vaccinia virus** infected MB49 tumor targets at effector to target ratios indicated. Percent specific lysis was calculated as follows: (cpm experimental release-cpm spontaneous release)/(total release-spontaneous release).times.100. Spleens of intravesically infected C57BL/6 mice were

tested for antigen specific killing of **vaccinia virus** infected MB-49 bladder tumor target cells in 4 hour chromium release assays. No **virus**-induced target lysis was seen in the 4 hour assay and **virus**-specific CTL did not lyse uninfected targets. As shown in FIG. 1, concentrations as low as 10 pfu intravesically were sufficient to induce a systemic anti-**vaccinia** CTL response. When the dose of intravesical **vaccinia** was titrated, concentrations of greater than 10.sup.5 pfu per mouse were lethal to nonimmunized mice, which died within 5-6 days. . . pfu appeared normal and survived greater than 2 weeks post-instillation. Mice made preimmune with an intraperitoneal injection of wild-type WR **vaccinia virus** (10.sup.7 pfu) demonstrated no morbidity at intravesical concentrations as high as 2.times.10.sup.6 pfu of **vaccinia** recombinants per mouse.

DETD C57BL/6 female mice were given a single intravesical instillation with **vaccinia** recombinant H1-VAC or NP-VAC (10.sup.4 pfu) to confirm infection of the urothelium. The mice were sacrificed, post instillation, and their. . . wall by routine pathology procedures using H & E stained slides demonstrated that urothelial cells lining the bladder lumen were **virus** infected as indicated by characteristic morphologic changes including cell enlargement, nuclear and cytoplasmic vacuolization, as well as atypical chromatin pattern.

DETD Human Study Using Intratumoral **Vaccinia** Injections as a Vector for Gene Transfer

DETD Patients in this study each had histologically documented, surgically incurable melanoma with at least one **dermal**, subcutaneous or lymph node metastasis which was evaluable for local response and accessible for injection. Eligible patients were fully ambulatory. . . eight weeks since chemotherapy or radiation therapy. All patients were immunocompetent as demonstrated by one or more positive cutaneous delayed-type **hypersensitivity** reactions to recall microbial antigens or to dinitrofluorobenzene after sensitization.

DETD Each patient was vaccinated, using a standard multipuncture method with a bifurcated vaccination needle, on the **skin** of the deltoid area which in all cases was a tumor free extremity with intact regional lymph nodes. The vaccination. . . a major local reaction (erythematous papule with vesiculation and pustule formation) was in progress. Tumor treatment commenced on day 4. **Dermal**, subcutaneous and/or lymph node metastases were infiltrated with wild-type **vaccinia virus** by intralesional injection using a 25 gauge needle (volume of injection ranged from 0.05 to 0.1 ml). Treatment was repeated. . .

DETD Punch biopsies were also performed using conventional sterile **dermatologic** techniques. One half of the material was fixed in formalin, paraffin embedded and sections stained with hematoxylin and eosin for. . . 5% fetal calf serum (FCS) and stained with the antibody TW2.3 which is specific for an early gene product of **vaccinia virus** replication (E3L). As E3L is a non-structural viral protein, positive antibody staining is indicative of active infection.

DETD To measure serum titers for anti-**vaccinia virus** antibody, ninety-six well plates were coated with a 10 .mu.g/ml protein extract obtained from cultures of human melanoma cell lines infected for 6 hours with the Wyeth strain of **vaccinia virus**. Following blocking with PBS plus FCS, dilution series of patient sera pre- and post-immunization were added to the wells, incubated for two

hours and the plates washed. Serum anti-**vaccinia virus** antibodies were visualized using a peroxidase labeled anti-human IgG heavy and light chain second reagent and orthophenyldiamine substrate. Titers were. . .

DETD Intralesional Infection of Human Melanoma Cells by **Vaccinia Virus**

DETD . . . (1 mm, level 4) of the right calf with satellite lesions in 1983. The primary lesion was excised and the **dermal** satellites successfully treated with intratumoral BCG. The patient did well until 1992 when two **dermal**/sc lesions appeared on the calf and failed to respond to intratumoral BCG, systemic R24 or chemotherapy. **Vaccinia** treatment was initiated with a standard immunization (250,000 pfu topically, 15 punctures). On day 4 of treatment, when it was determined that a take was clearly in progress, intralesional **vaccinia** was commenced. A single metastatic lesion was injected 19 times over 88 elapsed days with a total of 14.times.10.sup.7 pfu. . . (Wyeth). Several biopsies showed progressively intense infiltration of the tumor with lymphocytes and tumor regression. EM and immunohistological staining for **vaccinia** gene products showed successful viral infection of tumor cells in the presence of substantial anti-**vaccinia** antibody titers.

DETD . . . vaccine is demonstrated by a major reaction characterized by pustule formation at the vaccination site and the detection of circulating anti-**vaccinia** antibody. Patients exhibiting both response are eligible for localized treatment with the cytokine producing **vaccinia** vector.

DETD Patients are treated with increasing doses of the **vaccinia** over a several week period by local (intratumoral or **topical** such as intravesical) administration. In the case of melanoma, head and neck, and other tumors which grow as accessible solid masses at the primary and or metastatic sites, the **vaccinia** is injected into the tumor using a syringe and needle. In the case of bladder cancer, the **vaccinia** is instilled onto the bladder (intravesically) using a catheter.

CLM What is claimed is:

1. A method of expressing granulocyte-macrophage colony stimulating factor in tumors in situ comprising: a) generating a **vaccinia virus** vector encoding a gene for granulocyte-macrophage colony stimulating factor operably linked to transcriptional regulatory elements; and b) injecting said **vaccinia virus** into a tumor so that cells of the tumor express granulocyte-macrophage colony stimulating factor.

2. A method of producing regression of a tumor in a mammal having cancer comprising administering to the tumor in said mammal a **vaccinia virus** vector encoding a gene for granulocyte-macrophage colony stimulating factor operably linked to transcriptional regulatory elements in an amount capable of inducing an **immune response** to the tumor in the mammal such that the tumor regresses.

L13 ANSWER 7 OF 285 CAPLUS COPYRIGHT 2001 ACS

TI Vaccination by **topical** application of genetic vectors

PI WO 9908713 A1 **19990225**

PATENT NO. KIND DATE APPLICATION NO. DATE

PI	WO 9908713	A1	19990225	WO 1998-US16739	19980813 <--
	W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
	AU 9887807	A1	19990308	AU 1998-87807	19980813 <--
	EP 1015035	A1	20000705	EP 1998-939363	19980813
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
	JP 2001515052	T2	20010918	JP 2000-509449	19980813

AB The present invention provides a method of inducing an **immune response** in a non-invasive mode, comprising the step of: contacting **skin** of an individual in need of such treatment topically by applying to said **skin** an immunol. effective concn. of a genetic vector encoding a gene of interest. Also provided is a method of inducing an anti-tumor **immune response** in an animal in need of such treatment, comprising the step of: contacting **skin** of said animal topically by applying to said **skin** an immunol. effective concn. of a vector encoding a gene which encodes an antigen which induces an anti-tumor effect in. . . administration. The genetic vector may include adenovirus recombinants, DNA/adenovirus complexes, DNA/liposome complexes, or any other vectors capable of expressing transgenes. **Topical** application of genetic vectors may preferably include a device as designed therein.

IT Antigens
Interferons
Interleukin 12
Interleukin 2
RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(B7-2 gene; genetic vector encoding antigen gene and cytokine gene for **topical** vaccination)

IT Tetanus toxin
RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(C fragment; genetic vector encoding antigen gene and cytokine gene for **topical** vaccination)

IT Animal **virus**
Liposomes (drug delivery systems)
(DNA complex; genetic vector encoding antigen gene and cytokine gene for **topical** vaccination)

IT Medical goods
(adhesive sheet; genetic vector encoding antigen gene and cytokine gene for **topical** vaccination)

IT Dressings (medical)
(adhesive; genetic vector encoding antigen gene and cytokine gene for **topical** vaccination)

IT Immunomodulators
(gene; genetic vector encoding antigen gene and cytokine gene for **topical** vaccination)

IT CD80 (antigen)
Cytokines
Tumor-associated antigen
RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(gene; genetic vector encoding antigen gene and cytokine gene for **topical** vaccination)

IT Animal
Antitumor agents
Bandages
Genetic vectors
Human adenovirus
Human immunodeficiency **virus**
Influenza
Medical goods
Plasmids
Vaccines
Vertebrate (Vertebrata)
(genetic vector encoding antigen gene and cytokine gene for **topical** vaccination)

IT Carcinoembryonic antigen
Oncogenes
Tumor suppressor genes (animal)
gp120 (env glycoprotein)
RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(genetic vector encoding antigen gene and cytokine gene for **topical** vaccination)

IT DNA
Transgenes
RL: BPN (Biosynthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(genetic vector encoding antigen gene and cytokine gene for **topical** vaccination)

IT Polymers, biological studies
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(genetic vector encoding antigen gene and cytokine gene for **topical** vaccination)

IT **Hemagglutinins**
Protein NP (nucleoprotein)
RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(**influenza**; genetic vector encoding antigen gene and cytokine gene for **topical** vaccination)

IT Vaccination
(non-invasive **topical**; genetic vector encoding antigen gene and cytokine gene for **topical** vaccination)

IT Medical goods
(pads; genetic vector encoding antigen gene and cytokine gene for **topical** vaccination)

IT **Skin**
(vaccination; genetic vector encoding antigen gene and cytokine gene for **topical** vaccination)

IT Pathogen
Tumors (animal)
(vaccine; genetic vector encoding antigen gene and cytokine gene for **topical** vaccination)

IT 83869-56-1P, GM-CSF
RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(genetic vector encoding antigen gene and cytokine gene for **topical** vaccination)
IT 25104-18-1, Poly-L-lysine 38000-06-5, Poly-L-lysine
RL: BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(genetic vector encoding antigen gene and cytokine gene for **topical** vaccination)

L13 ANSWER 8 OF 285 CAPLUS COPYRIGHT 2001 ACS
TI Vaccination of mammals and birds using DNA encoding IL6 and an **immune response**-inducing antigen
PI WO 9904009 A1 **19990128**

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE	
PI WO 9904009	A1	19990128	WO 1998-US14334	19980710	<--
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM					
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG					
AU 9883928	A1	19990210	AU 1998-83928	19980710	<--
US 2001007860	A1	20010712	US 1998-113836	19980710	

AB A method of providing a patient with an enhanced **immune response** is disclosed comprising the step of vaccinating the patient with a vaccine comprising a combination of DNA encoding interleukin-6 and DNA encoding an antigen capable of enlisting an enhanced **immune response** in a patient. In one embodiment, the enhanced **immune response** is a therapeutic response. In another embodiment, the enhanced **immune response** is a protective **immune response**.
IT Surface antigens
RL: BPN (Biosynthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(human **hepatitis**; method of vaccination using DNA encoding IL6 and an **immune response**-inducing antigen)
IT T cell (lymphocyte)
(**immune response**; method of vaccination using DNA encoding IL6 and an **immune response**-inducing antigen)
IT Antibodies
RL: MFM (Metabolic formation); BIOL (Biological study); FORM (Formation, nonpreparative)
(**immune response**; method of vaccination using DNA encoding IL6 and an **immune response**-inducing antigen)
IT Equine **influenza virus**
Gene therapy
Genetic vectors
Horse (Equus caballus)
Mammal (Mammalia)
Microprojectile bombardment
Mouse

Swine **influenza virus**
Transformation (genetic)
(method of vaccination using DNA encoding IL6 and an **immune response**-inducing antigen)

IT **Hemagglutinins**
Interleukin 6
Tumor-associated antigen
RL: BPN (Biosynthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(method of vaccination using DNA encoding IL6 and an **immune response**-inducing antigen)

IT Mucosal immunity
Skin
Tongue
(particle bombardment transformation; method of vaccination using DNA encoding IL6 and an **immune response**-inducing antigen)

IT Vaccines
(prophylactic and therapeutic; method of vaccination using DNA encoding
IL6 and an **immune response**-inducing antigen)

IT Immunity
(protective; method of vaccination using DNA encoding IL6 and an **immune response**-inducing antigen)

IT **Hepatitis virus**
Herpesviridae
Influenza virus
Rotavirus
(vaccination against; method of vaccination using DNA encoding IL6 and an **immune response**-inducing antigen)

IT Antigens
RL: BPN (Biosynthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(viral and bacterial; method of vaccination using DNA encoding IL6 and an **immune response**-inducing antigen)

L13 ANSWER 9 OF 285 USPATFULL
PI US 6008035 19991228 <--
AB . . . tissue, and viral genomes persist in bone marrow for at least three months post-infection. No or very low levels of **virus** were detected in quadricep, brain, and sera of treated animals. The sequence of a consensus Sindbis cDNA clone, pTR339, and infectious RNA transcripts, infectious **virus** particles, and pharmaceutical formulations derived therefrom are also disclosed. The sequence of the genomic RNA of the Girdwood S.A. **virus**, and cDNA clones, infectious RNA transcripts, infectious **virus** particles, and pharmaceutical formulations derived therefrom are also disclosed.

SUMM The Alphavirus genus includes a variety of **viruses** all of which are members of the Togaviridae family. The alphaviruses include Eastern Equine Encephalitis **virus** (EEE), Venezuelan Equine Encephalitis **virus** (VEE), Everglades **virus**, Mucambo **virus**, Pixuna **virus**, Western Equine Encephalitis **virus** (WEE), Sindbis **virus**, South African Arbovirus No. 86 (S.A.AR 86), Girdwood S.A. **virus**, Ockelbo **virus** , Semliki Forest **virus**, Middelburg **virus**, Chikungunya **virus**, O'Nyong-Nyong **virus**, Ross River **virus**, Barmah Forest **virus**, Getah **virus**, Sagiyama **virus**, Bebaru **virus**, Mayaro **virus** , Una **virus**, Aura **virus**, Whataroa **virus**, Babanki **virus**, Kyzylagach **virus**, Highlands J

virus, Fort Morgan **virus**, Ndumu **virus**, and Buggy Creek **virus**.

SUMM Sindbis **virus**, the prototype member of the alphavirus genus of the family Togaviridae, and **viruses** related to Sindbis are broadly distributed throughout Africa, Europe, Asia, the Indian subcontinent, and Australia, based on serological surveys of. . . Epidemiol. Infect. 106, 567-74 (1991); Morrill et al., J. Trop. Med. Hyg. 94, 166-68 (1991). The first isolate of Sindbis **virus** (strain AR339) was recovered from a pool of Culex sp. mosquitoes collected in Sindbis, Egypt in 1953 (Taylor et al.,. . . group. Other members of the Sindbis group of alphaviruses include South African Arbovirus No. 86, Ockelbo82, and Girdwood S.A. These **viruses** are not strains of the Sindbis **virus**; they are related to Sindbis AR339, but they are more closely related to each other based on nucleotide sequence and. . . not. The clinical symptoms of human infection with Ockelbo82, S.A.AR86, or Girdwood S.A. are a febrile illness, general malaise, macropapular **rash**, and joint pain that occasionally progresses to a polyarthralgia sometimes lasting from a few months to a few years.

SUMM The study of these **viruses** has led to the development of beneficial techniques for vaccinating against the alphavirus diseases, and other diseases through the use. . .

SUMM . . . well known that live, attenuated viral vaccines are among the most successful means of controlling viral disease. However, for some **virus** pathogens, immunization with a live **virus** strain may be either impractical or unsafe. One alternative strategy is the insertion of sequences encoding immunizing antigens of such agents into a vaccine strain of another **virus**. One such system utilizing a live VEE vector is described in U.S. Pat. No. 5,505,947 to Johnston et al.

SUMM Sindbis **virus** vaccines have been employed as viral carriers in **virus** constructs which express genes encoding immunizing antigens for other **viruses**. See U.S. Pat. No. 5,217,879 to Huang et al. Huang et al. describes Sindbis infectious viral vectors. However, the reference. . .

SUMM Another such system is described by Hahn et al., Proc. Natl. Acad. Sci. USA 89:2679 (1992), wherein Sindbis **virus** constructs which express a truncated form of the **influenza hemagglutinin** protein are described. The constructs are used to study antigen processing and presentation in vitro and in mice. Although no. . .

SUMM London et al., Proc. Natl. Acad. Sci, USA 89, 207-11 (1992), disclose a method of producing an **immune response** in mice against a lethal Rift Valley Fever (RVF) **virus** by infecting the mice with an infectious Sindbis **virus** containing an RVF epitope. London does not disclose using Girdwood S.A. or TR339 to induce an **immune response** in animals.

SUMM . . . system for introducing and expressing foreign proteins in animal cells using alphaviruses. This reference discloses the use of Semliki Forest **virus** to introduce and express foreign proteins in animal cells. The use of Girdwood S.A. or TR339 is not discussed. Furthermore,. . .

SUMM Accordingly, there remains a need in the art for full-length cDNA clones of positive-strand RNA **viruses**, such as Girdwood S.A and TR339. In addition, there is an ongoing need in the art for improved vaccination strategies.. . .

SUMM As a second aspect, the present invention provides a helper cell for expressing an infectious, propagation defective, Girdwood S.A. **virus** particle, comprising, in a Girdwood S.A.-permissive cell: (a) a first helper RNA encoding (i) at least one Girdwood S.A. structural. . .

SUMM A third aspect of the present invention is a method of making infectious, propagation defective, Girdwood S.A. **virus** particles, comprising: transfecting a Girdwood S.A.-permissive cell with a propagation defective replicon RNA, the replicon RNA including the Girdwood S.A. packaging segment and an inserted heterologous RNA; producing the Girdwood S.A. **virus** particles in the transfected cell; and then collecting the Girdwood S.A. **virus** particles from the cell. Also disclosed are infectious Girdwood S.A. RNAs, cDNAs encoding the same, infectious Girdwood S.A. **virus** particles, and pharmaceutical formulations thereof.

SUMM As a fourth aspect, the present invention provides a helper cell for expressing an infectious, propagation defective, TR339 **virus** particle, comprising, in a TR339-permissive cell: (a) a first helper RNA encoding (i) at least one TR339 structural protein, and. . .

SUMM A fifth aspect of the present invention is a method of making infectious, propagation defective, TR339 **virus** particles, comprising: transfecting a TR339-permissive cell with a propagation defective replicon RNA, the replicon RNA including the TR339 packaging segment and an inserted heterologous RNA; producing the TR339 **virus** particles in the transfected cell; and then collecting the TR339 **virus** particles from the cell. Also disclosed are infectious TR339 RNAs, cDNAs encoding the same, infectious TR339 **virus** particles, and pharmaceutical formulations thereof.

SUMM As a sixth aspect, the present invention provides a recombinant DNA comprising a cDNA coding for an infectious Girdwood S.A. **virus** RNA transcript, and a heterologous promoter positioned upstream from the cDNA and operatively associated therewith. The present invention also provides. . .

SUMM . . . "alphavirus" has its conventional meaning in the art, and includes the various species of alphaviruses such as Eastern Equine Encephalitis **virus** (EEE), Venezuelan Equine Encephalitis **virus** (VEE), Everglades **virus**, Mucambo **virus**, Pixuna **virus**, Western Encephalitis **virus** (WEE), Sindbis **virus**, South African Arbovirus No. 86, Girdwood S.A. **virus**, Ockelbo **virus**, Semliki Forest **virus**, Middelburg **virus**, Chikungunya **virus**, O'Nyong-Nyong **virus**, Ross River **virus**, Barmah Forest **virus**, Getah **virus**, Sagiyama **virus**, Bebaru **virus**, Mayaro **virus**, Una **virus**, Aura **virus**, Whataroa **virus**, Babanki **virus**, Kyzylagach **virus**, Highlands J **virus**, Fort Morgan **virus**, Ndumu **virus**, Buggy Creek **virus**, and any other **virus** classified by the International Committee on Taxonomy of **Viruses** (ICTV) as an alphavirus. The preferred alphaviruses for use in the present invention include Sindbis **virus** strains (e.g., TR339), Girdwood S.A., S.A.AR86, and Ockelbo82.

SUMM An "Old World alphavirus" is a **virus** that is primarily distributed throughout the Old World. Alternately stated, an Old World alphavirus is a **virus** that is primarily distributed throughout Africa, Asia, Australia and New Zealand, or Europe. Exemplary Old World **viruses** include SF group alphaviruses and SIN group alphaviruses. SF group alphaviruses include Semliki Forest **virus**

, Middelburg **virus**, Chikungunya **virus**, O'Nyong-Nyong **virus**, Ross River **virus**, Barmah Forest **virus**, Getah **virus**, Sagiyama **virus**, Bebaru **virus**, Mayaro **virus**, and Una **virus**. SIN group alphaviruses include Sindbis **virus**, South African Arbovirus No. 86, Ockelbo **virus**, Girdwood S.A. **virus**, Aura **virus**, Whataroa **virus**, Babanki **virus**, and Kyzylagach **virus**.

SUMM . . . 132 (3d ed. 1980). The phrase "attenuating mutation" excludes mutations or combinations of mutations which would be lethal to the **virus**.

SUMM . . . is suitable. Preferred alphaviruses include Old World alphaviruses, more preferably SF group alphaviruses and SIN group alphaviruses, more preferably Sindbis **virus** strains (e.g., TR339), S.A.AR86 **virus**, Girdwood S.A. **virus**, and Ockelbo **virus**. In a more preferred embodiment, the alphavirus contains one or more attenuating mutations, as described hereinabove.

SUMM Two types of recombinant **virus** vector are contemplated in carrying out the present invention. In one embodiment employing "double promoter vectors," the heterologous RNA is inserted into a replication and propagation competent **virus**. Double promoter vectors are described in U.S. Pat. No. 5,505,947 to Johnston et al. With this type of viral vector,. . .

SUMM By targeting to the cells of the bone marrow, it is meant that the primary site in which the **virus** will be localized in vivo is the cells of the bone marrow. Alternately stated, the alphaviruses of the present invention. . . crushed bone, more preferably greater than 300 PFU/g crushed bone, and more preferably still greater than 500 PFU/g crushed bone. **Virus** may be detected occasionally in other cell or tissue types, but only sporadically and usually at low levels. **Virus** localization in the bone marrow can be demonstrated by any suitable technique known in the art, such as in situ. . .

SUMM . . . disease, including but not limited to microbial, bacterial, protozoal, parasitic, and viral diseases. For example, the immunogen may be an **orthomyxovirus** immunogen (e.g., an **influenza virus** immunogen, such as the **influenza virus hemagglutinin** (HA) surface protein or the **influenza virus** nucleoprotein gene, or an equine **influenza virus** immunogen), or a lentivirus immunogen (e.g., an equine infectious anemia **virus** immunogen, a Simian Immunodeficiency **Virus** (SIV) immunogen, or a Human Immunodeficiency **Virus** (HIV) immunogen, such as the HIV envelope GP160 protein and the HIV matrix/capsid proteins). The immunogen may also be an arenavirus immunogen (e.g., Lassa fever **virus** immunogen, such as the Lassa fever **virus** nucleocapsid protein gene and the Lassa fever envelope glycoprotein gene), a poxvirus immunogen (e.g., **vaccinia**), a flavivirus immunogen (e.g., a yellow fever **virus** immunogen or a Japanese encephalitis **virus** immunogen), a filovirus immunogen (e.g., an Ebola **virus** immunogen, or a Marburg **virus** immunogen), a bunyavirus immunogen (e.g., RVFV, CCHF, and SFS **viruses**), or a coronavirus immunogen (e.g., an infectious human coronavirus immunogen, such as the human coronavirus envelope glycoprotein gene, or a transmissible gastroenteritis **virus** immunogen for pigs, or an infectious bronchitis **virus** immunogen for chickens).

SUMM . . . the particular target being bound. The only limits on the length of the antisense oligonucleotide is the capacity of the

virus for inserted heterologous RNA. Antisense oligonucleotides may be complementary to the entire mRNA transcript of the target gene or only. . .

SUMM . . . are well known in the art. The Sindbis 26S promoter is preferred when the alphavirus is a strain of Sindbis **virus**. Additional preferred promoters beyond the Sindbis 26S promoter include the Girdwood S.A. 26S promoter when the alphavirus is Girdwood S.A.,. . .

SUMM . . . mucus membranes of a subject (e.g., intranasal administration, by use of a dropper, swab, or inhaler). Methods of preparing infectious **virus** particles and pharmaceutical formulations thereof are discussed in more detail hereinbelow.

SUMM . . . cDNAs encoding the same. Preferably the infectious RNAs and cDNAs are derived from the S.A.AR86, Girdwood S.A., TR339, or Ockelbo **viruses**. The cDNA clones can be generated by any of a variety of suitable methods known to those skilled in the. . .

SUMM . . . invention, double promoter vectors are used to introduce the heterologous RNA into the target bone marrow cells. A double promoter **virus** vector is a replication and propagation competent **virus**. Double promoter vectors are described in U.S. Pat. No. 5,505,947 to Johnston et al., the disclosure of which is incorporated. . . in its entirety by reference. Preferred alphaviruses for constructing the double promoter vectors are S.A.AR86, Girdwood S.A., TR339 and Ockelbo **viruses**. More preferably, the double promoter vector contains one or more attenuating mutations. Attenuating mutations are described in more detail hereinabove.

SUMM . . . double promoter vector is constructed so as to contain a second subgenomic promoter (i.e., 26S promoter) inserted 3' to the **virus** RNA encoding the structural proteins. The heterologous RNA is inserted between the second subgenomic promoter, so as to be operatively associated therewith, and the 3' UTR of the **virus** genome. Heterologous RNA sequences of less than 3 kilobases, more preferably those less than 2 kilobases, and more preferably still. . .

SUMM Replicon vectors, which are propagation-defective **virus** vectors can also be used to carry out the present invention. Replicon vectors are described in more detail in U.S.. . .

SUMM . . . translated to produce high levels of the foreign protein. The autonomously replicating RNA (i.e., replicon) can only be packaged into **virus** particles if the alphavirus structural protein genes are provided on one or more "helper" RNAs, which are cotransfected into cells. . . protein genes on the helper RNA, resulting in the synthesis of viral structural proteins and packaging of the replicon into **virus**-like particles. As the packaging or encapsidation signal for alphavirus RNAs is located within the nonstructural genes, the absence of these sequences in the helper RNAs precludes their incorporation into **virus** particles.

SUMM . . . and expression of two different heterologous proteins. Another useful strategy is to insert the IRES sequence from the picornavirus, EMC **virus**, between the two heterologous genes downstream from the single 26S promoter of the replicon described above, thus leading to expression. . .

SUMM The alphavirus vectors, RNAs, cDNAs, helper cells, infectious **virus** particles, and methods of the present invention find use in in vitro expression systems, wherein the inserted heterologous RNA encodes a protein or peptide which is desirably produced in vitro. The

RNAs, cDNAs, helper cells, infectious **virus** particles, methods, and pharmaceutical formulations of the present invention are additionally useful in a method of administering a protein or. . .

SUMM Disclosed hereinbelow are genomic RNA sequences encoding live Girdwood S.A. **virus**, live S.A.AR86 **virus**, and live Sindbis strain TR339 **virus**, cDNAs derived therefrom, infectious RNA transcripts encoded by the cDNAs, infectious viral particles containing the infectious RNA transcripts, and pharmaceutical. . .

SUMM The cDNAs encoding infectious Girdwood S.A. and TR339 **virus** RNA transcripts of the present invention include those homologous to, and having essentially the same biological properties as, the cDNA. . . SEQ ID NO:4 and SEQ ID NO:8, respectively. Thus, cDNAs that hybridize to cDNAs encoding infectious Girdwood S.A. or TR339 **virus** RNA transcripts disclosed herein are also an aspect of this invention. Conditions which will permit other cDNAs encoding infectious Girdwood S.A. or TR339 **virus** transcripts to hybridize to the cDNAs disclosed herein can be determined in accordance with known techniques. For example, hybridization of. . . 5.times. Denhardt's solution, 0.5% SDS and 1.times. SSPE at 42.degree. C., respectively, to cDNA encoding infectious Girdwood S.A. or TR339 **virus** RNA transcripts disclosed herein in a standard hybridization assay. See J. SAMBROOK ET AL., MOLECULAR CLONING: A LABORATORY MANUAL (2d ed. 1989)). In general, cDNA sequences encoding infectious Girdwood S.A. or TR339 **virus** RNA transcripts that hybridize to the cDNAs disclosed herein will be at least 30% homologous, 50% homologous, 75% homologous, and even 95% homologous or more with the cDNA sequences encoding infectious Girdwood S.A. or TR339 **virus** RNA transcripts disclosed herein.

SUMM Promoter sequences and Girdwood S.A. **virus** or Sindbis **virus** strain TR339 cDNA clones are operatively associated in the present invention such that the promoter causes the cDNA clone to. . .

SUMM The Girdwood S.A. and TR339 cDNA clones and the infectious RNAs and infectious **virus** particles produced therefrom of the present invention are useful for the preparation of pharmaceutical formulations, such as vaccines. In addition,. . . of the present invention are useful for administration to animals for the purpose of producing antibodies to the Girdwood S.A. **virus** or the Sindbis **virus** strain TR339, which antibodies may be collected and used in known diagnostic techniques for the detection of Girdwood S.A. **virus** or Sindbis **virus** strain TR339. Antibodies can also be generated to the viral proteins expressed from the cDNAs disclosed herein. As another aspect. . .

SUMM III. Infectious **Virus** Particles and Pharmaceutical Formulations

SUMM The infectious **virus** particles of the present invention include those containing double promoter vectors and those containing replicon vectors as described hereinabove. Alternately, the infectious **virus** particles contain infectious RNAs encoding the Girdwood S.A. or TR339 genome. When the infectious RNA comprises the Girdwood S.A. genome,. . .

SUMM . . . out according to any suitable means known to those skilled in the art, as described above with respect to propagation-competent **viruses**.

SUMM The step of collecting the infectious **virus** particles may also be carried out using conventional techniques. For example, the infectious particles may be collected by cell lysis,. . . to Temin et

al. Other suitable techniques will be known to those skilled in the art.

Optionally, the collected infectious **virus** particles may be purified if desired. Suitable purification techniques are well known to those skilled in the art.

SUMM Pharmaceutical formulations, such as vaccines, of the present invention comprise an immunogenic amount of the infectious, **virus** particles in combination with a pharmaceutically acceptable carrier. An "immunogenic amount" is an amount of the infectious **virus** particles which is sufficient to evoke an **immune response** in the subject to which the pharmaceutical formulation is administered. An amount of from about 10.sup.3 to about 10.sup.7 particles,. . . is believed suitable, depending upon the age and species of the subject being treated, and the immunogen against which the **immune response** is desired.

SUMM Pharmaceutical formulations of the present invention for therapeutic use

comprise a therapeutic amount of the infectious **virus** particles in combination with a pharmaceutically acceptable carrier. A "therapeutic amount" is an amount of the infectious **virus** particles which is sufficient to produce a therapeutic effect (e.g., triggering an **immune response** or supplying a protein to a subject in need thereof) in the subject to which the pharmaceutical

formulation is administered.. . .

SUMM . . . to, sterile pyrogen-free water and sterile pyrogen-free physiological saline solution. Subjects which may be administered immunogenic amounts of the infectious **virus** particles of the present invention include but are not limited to human and animal (e.g.,

pig, cattle, dog, horse, donkey,. . .

DETD Cells and **Virus** Stocks

DETD . . . the Johannesburg area of South Africa in 1963. Malherbe et al.,

S. Afr. Med. J. 37, 547-52 (1963). Molecularly cloned **virus** TR339 represents the deduced consensus sequence of Sindbis AR339. McKnight et al., J. Virol. 70, 1981-89 (1996); William Klimstra, personal. . .

DETD Stocks of all molecularly cloned **viruses** were prepared by electroporating genome length in vitro transcripts of their respective cDNA clones in BHK-21 cells. Heidner et al.,. . . Hyg. 33, 1212-17 (1984)) were passed one to three times in BHK-21 cells in order to produce amplified stocks of **virus**. All **virus** stocks were stored at -70.degree. C. until needed. The titers of the **virus** stocks were determined on BHK-21 cells from aliquots of frozen **virus**.

DETD . . . the alphavirus prototype Sindbis strain AR339. Strauss et al., Virology 133, 92-110 (1984). Compared with the consensus sequence of Sindbis **virus** AR339 (McKnight et al., J. Virol. 70 1981-89 (1996)), S.A.AR86 contained two separate 6-nucleotide insertions, and one 3-nucleotide insertion in. . .

DETD Comparison of S.A.AR86 and Girdwood S.A. Sequences With Other Sindbis-Related **Virus** Sequences

DETD Table 1 examines the relationship of S.A.AR86 and Girdwood S.A. to each other and to other Sindbis-related **viruses**. This was accomplished by aligning the nucleotide and deduced amino acid sequences

of Ockelbo82, AR339 and Girdwood S.A. to those. . .

DETD . . . than to the Egyptian Sindbis AR339 isolate. These results also suggest that it is unlikely that S.A.AR86 is a recombinant **virus**

like WEE **virus**. Hahn et al., Proc. Natl. Acad. Sci. USA 85, 5997-6001 (1988).

DETD TABLE 1

Comparison of the Nucleotide and Amino Add Sequences of S.A.AR86 **Virus** with Those of Sindbis AR339, Ockelbo82, and Girdwood

S.A. **Viruses**.sup.a

	Nucleotide Differences.sup.b		Amino Acid Differences.sup.b	
	AR339	OCK82	AR339	OCK82
Regions	Number (%)	GIRD Number (%)		GIRD
5' untranslated	0 (0.0)	0 (0.0)		

. . .

DETD . . . phenotype if the S.A.AR86 amino acid differed from that which otherwise was absolutely conserved at that position in the other **viruses**.

DETD As has been described by Simpson et al., Virology 222, 464-69 (1996), neurovirulence and replication of the **virus** derived from pS55 (S55) were compared with those of S.A.AR86. It was found that S55 exhibits the distinctive adult neurovirulence characteristic of S.A.AR86. Like S.A.AR86, S55 produces 100% mortality in adult mice infected with the **virus** and the survival times of animals infected with both **viruses** were indistinguishable. In addition, S55 and S.A.AR86 were found to replicate to essentially equivalent titers in vivo, and the profiles of S55 and S.A.AR86 **virus** growth in the central nervous system and periphery were very similar.

DETD From these data it was concluded that the silent changes found in **virus** derived from clone pS55 had little or no effect on its growth or virulence, and that this molecularly cloned **virus** accurately represents the biological isolate, S.A.AR86.

DETD Construction of the Consensus AR339 **Virus** TR339

DETD The consensus sequence of the Sindbis **virus** AR339 isolate, the prototype alphavirus was deduced. The consensus AR339 sequence was inferred by comparison of the TRSB sequence (a. . . AR339 strain; Davis et al., Virology 161, 101-108 (1987), Strauss et al., J. Virol. 65, 4654-64 (1991)). Each of these **viruses** was descended from AR339. Where these sequences differed from each other, they also were compared with the amino acid sequences of other **viruses** related to Sindbis **virus**: Ockelbo82, S.A.AR86, Girdwood S.A., and the somewhat more distantly related Aura **virus**. Rumenapf et al., Virology 208, 621-33 (1995).

DETD The details of determining a consensus AR339 sequence and constructing the consensus **virus** TR339 have been described elsewhere. McKnight et al., J. Virol. 70, 1981-89 (1996); Klimstra et al., manuscript in preparation. The. . .

DETD . . . donor calf serum, 100 u/ml penicillin, 50 .mu.g/ml streptomycin, 0.9 mM CaCl.sub.2, and 0.5 mM MgCl.sub.2) containing 10.sup.3 PFU of **virus** were injected either intravenously (iv) into the tail vein, subcutaneously (sc) into the **skin** above the shoulder blades on the middle of the back, or intraperitoneally (ip) in the lower right abdomen. Animals were. . .

DETD . . . length of which 445 nucleotides were complementary to the S.A.AR86 genome (nucleotides 7371 through 7816). A riboprobe specific for the **influenza** strain PR-8 **hemagglutinin** (HA) gene was used as a control probe to test non-specific binding. The in situ hybridizations were performed as described. . .

DETD . . . post-inoculation by exsanguination. The serum, brain (including brainstem), right quadricep, and both femurs were harvested and titered by plaque assay. **Virus** was never detected in the quadricep samples of animals inoculated sc (Table 4). A single animal inoculated ip (two days post-inoculation) and two mice inoculated iv (at four and six days post-inoculation) had detectable **virus** in the right quadricep, but the titer was at or just above the limit of detection (6.25 PFU/g tissue). **Virus** was present sporadically or at low levels in the brain and serum of animals regardless of the route of inoculation. **Virus** was detected in the bone marrow of animals regardless of the route of inoculation. However, the presence of **virus** in bone marrow of animals inoculated sc or ip was more sporadic than animals inoculated iv, where five out of six animals had detectable **virus**. These results suggest that S55 targets to the bone marrow, especially following iv inoculation.

DETD The level and frequency of **virus** detected in the serum and muscle suggested that **virus** detected in the bone marrow was not residual **virus** contamination from blood or connective tissue remaining in bone marrow samples. The following experiment also suggested that **virus** in bone marrow was not due to tissue or serum contamination. Mice were inoculated ic with 1200 PFU of S55. .

.

DETD Other Sindbis Group **Viruses**

DETD . . . ability to replicate in the bone marrow of mice was unique to S55 or was a general feature of other **viruses**, both Sindbis and non-Sindbis **viruses**, in the Sindbis group. Six 38-day-old female CD-1 mice were inoculated iv with 25 .mu.l of diluent containing 10.sup.3 PFU. . . from each group were sacrificed and whole blood, serum, brain (including brainstem), right quadricep, and both femurs were harvested for **virus** titration.

DETD The results of this experiment were similar to those with S55. TRSB infected animals had no **virus** detectable in serum or whole blood in any animal at any time, and with the other **viruses** tested, no **virus** was detected in the serum or whole blood of any animal beyond two days post-inoculation (detection limit, 25 PFU/ml). Neither. . . of infected animals sporadically with the titers being at or near the 75 PFU/g level of detection. All the tested **viruses** were found sporadically at or slightly above the 50 PFU/g detection limit in the right quadricep of infected animals except for a single animal four days post-inoculation with TRSB which had nearly 10.sup.5 PFU/g of **virus** in its quadricep.

DETD The frequency at which the different **viruses** were detected in bone marrow varied widely, with S55 and Girdwood S.A. being the most frequently isolated (five out of. . . not neurovirulent in adult mice (Example 4), suggesting that the adult neurovirulence phenotype is distinct from the ability of the **virus** to replicate efficiently in bone marrow.

DETD TABLE 4

Titers Following IV Inoculation of **Virus**

Tissue Titered

Bone Marrow

Serum

Virus	Animal	Days Post-Inoculation	Blood (PFU/g)	Brain (PFU/ml)	Quadricep (PFU/ml) (PFU/g)
S55	A	2	1125	N.D..sup.a	N.D. N.D. N.D.
	B	488 50 200	N.D.. . .	N.D.	
	B	N.D. N.D. N.D.	N.D. N.D.		
	Limit of Detection		37.5 25 25	75 50	

.sup.a "N.D." indicates that the **virus** titers were below the limit of detection.

DETD **Virus** Persistence in Bone Marrow

DETD . . . 4, 8, 16, and 30 days post-inoculation for determination of bone marrow and serum titers. At no time post-inoculation was **virus** detected in the serum above the 6.25 PFU/ml detection limit. **Virus** was detectable in the bone marrow samples of both animals sampled at four days post-inoculation and in one animal eight days post-inoculation (Table 5). No **virus** was detectable by sitration on BHK-21 cells in any of the bone marrow samples beyond eight days post-inoculation. These results suggested that the attenuating mutation present in S51, which reduces the neurovirulence of the **virus**, did not impair acute viral replication in the bone marrow.

DETD It was notable that the plaque size on BHK-21 cells of **virus** recovered on day 4 post-inoculation was smaller than the size of plaques produced by the inoculum **virus**, and that plaques produced from **virus** recovered from the day 8 post-inoculation samples were even smaller and barely visible. This suggests a strong selective pressure in the bone marrow for **virus** that is much less efficient in forming plaques on BHK-21 cells.

DETD To demonstrate that S51 **virus** genomes were present in bone marrow cells long after acute infection, four to six-week-old female CD-1 mice were inoculated ic. . . legs (data not shown). Furthermore, no in situ hybridization signal was detected in an adjacent control section probed with an **influenza virus** HA gene specific riboprobe. As the relative sensitivity of in situ hybridization is reduced in decalcified tissues (Peter Charles, personal. . .

DETD . . . 380 62.5

8	62.5	.sup.	N.D..sup.a 62.5
16	N.D.	N.D.	62.5
30	N.D.	N.D.	62.5

.sup.a "N.D." indicates that the **virus** titers were below the limit of detection.

CLM What is claimed is:

1. A helper cell for expressing an infectious, propagation defective, TR339 **virus** particle, comprising, in a TR339-permissive cell: (a) a first helper RNA encoding (i) at least one TR339 structural protein, and. . .

23. A method of making infectious, propagation defective, TR339 **virus** particles, comprising: transfecting a TR339-permissive cell according to claim 1 with a propagation defective replicon RNA, said replicon RNA including an alphavirus packaging segment and an inserted heterologous RNA; producing said TR339 **virus** particles in said transfected cell; and then collecting said TR339 **virus** particles from said cell.

24. Infectious TR339 **virus** particles produced by the method of claim 23.

25. A pharmaceutical formulation comprising infectious TR339 **virus** particles according to claim 24 in a pharmaceutically acceptable carrier.

26. The pharmaceutical formulation according to claim 25, wherein said infectious TR339 **virus** particles are included therein in an immunogenic amount.

27. Infectious TR339 **virus** particles containing a replicon RNA encoding a promoter, an inserted heterologous RNA, and wherein RNA encoding at least one TR339 structural protein is deleted therefrom so that said **virus** particle is propagation defective.

39. A composition comprising infectious, propagation defective, **virus** particles, wherein each particle comprises a TR339 replicon RNA, and wherein the replicon RNA comprises an alphavirus packaging signal, one. . .
42. A composition comprising infectious, propagation defective, **virus** particles, wherein each particle comprises a TR339 nucleocapsid and an alphavirus replicon RNA, wherein the replicon RNA comprises an alphavirus. . .
46. A composition comprising infectious, propagation defective, **virus** particles, wherein each particle comprises an alphavirus nucleocapsid and a TR339 replicon RNA, wherein the TR339 replicon RNA comprises an. . .
49. A cDNA encoding a TR339 **virus** genome.

51. An RNA comprising a TR339 **virus** genome.

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DETD . . . fused to the marker in the case of a bacterial host, or, for example, the marker sequence may be a **hemagglutinin** (HA) tag when a mammalian host, e.g. COS-7 cells, is used. The HA tag corresponds to an epitope derived from the **influenza hemagglutinin** protein (Wilson, I., et al., Cell, 37:767 (1984)).
DETD . . . bacterial plasmids; phage DNA; baculovirus; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA such as **vaccinia**, adenovirus, fowl pox **virus**, and pseudorabies. However, any other vector may be used as long as it is replicable and viable in the host.
DETD . . . phage lambda P.sub.L promoter and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their **viruses**. The expression vector also contains a ribosome binding site for translation initiation and a transcription terminator. The vector may also. . .
DETD . . . resulting from trauma such as burns, abrasions and cuts, as

well as from surgical procedures such as surgical incisions and **skin** grafting. Other conditions suitable for treatment with the polypeptide of the present invention include chronic conditions, such as chronic ulcers,. . .

DETD . . . nature of the carriers may vary widely and will depend on the intended location of application. For application to the **skin**, a cream or ointment base is usually preferred; suitable bases include lanolin, Silvadene (Marion) (particularly for the treatment of burns),. . .

DETD . . . topically to the affected area, typically as eye drops to the eye or as creams, ointments or lotions to the **skin**. In the case of the eyes, frequent treatment is desirable, usually being applied at intervals of 4 hours or less. On the **skin**, it is desirable to continually maintain the treatment composition on the affected area during the healing, with applications of the. . .

DETD . . . T helper 1 cells. Thus, it is believed tht HTTER36 may be used as a therapeutic to ehance the cellular **immune response**.

DETD . . . is believed the HTTER36 polypeptides may be used to treat inflammatory diseases such as, Lyme's disease, arthritis, reactive arthritis, contact **dermatitis** as well as malaria-induced vascular disease. HTTER36 may also be useful in the treatment of autoimmune diseases such as multiple. . .

DETD The pharmaceutical compositions may be administered in a convenient manner such as by the oral, **topical**, intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal or intradermal routes. The pharmaceutical compositions are administered in an amount which is effective for. . .

DETD . . . from which the retroviral plasmid vectors hereinabove mentioned may be derived include, but are not limited to, Moloney Murine Leukemia **Virus**, spleen necrosis **virus**, retroviruses such as Rous Sarcoma **Virus**, Harvey Sarcoma **Virus**, avian leukosis **virus**, gibbon ape leukemia **virus**, human immunodeficiency **virus**, adenovirus, Myeloproliferative Sarcoma **Virus**, and mammary tumor **virus**. In one embodiment, the retroviral plasmid vector is derived from Moloney Murine Leukemia **Virus**.

DETD . . . promoters, such as the adenoviral major late promoter; or heterologous promoters, such as the cytomegalovirus (CMV) promoter; the respiratory syncytial **virus** (RSV) promoter; inducible promoters, such as the MMT promoter, the metallothionein promoter; heat shock promoters; the albumin promoter; the ApoAI promoter; human globin promoters; viral thymidine kinase promoters, such as the **Herpes** Simplex thymidine kinase promoter; retroviral LTRs (including the modified retroviral LTRs hereinabove described); the .beta.-actin promoter; and human growth hormone. . .

DETD . . . of the proteins compared to normal control tissue samples can detect the presence of certain disease conditions such as neoplasia, **skin** disorders, ocular disorders and inflammation. Assays used to detect levels of the polypeptide of the present invention in a sample. . .

DETD . . . Agricultural Experimental Station Bulletin No. 1555). This expression vector contains the strong polyhedrin promoter of the Autographa californica nuclear polyhedrosis **virus** (AcMNPV) followed by the recognition sites for the restriction endonucleases. The polyadenylation site of the simian **virus** (SV)40 is used for

efficient polyadenylation. For an easy selection of recombinant **virus** the beta-galactosidase gene from E.coli is inserted in the same orientation as the polyhedrin promoter followed by the polyadenylation signal. . .

DETD 1 .mu.g of BaculoGold.TM. **virus** DNA and 5 .mu.g of the plasmid pBacHTTER36 are mixed in a sterile well of a microtiter plate containing 50. . .

DETD Four days after the serial dilution, the **virus** is added to the cells and blue stained plaques are picked with the tip of an Eppendorf pipette. The agar containing the recombinant **viruses** is then resuspended in an Eppendorf tube containing 200 .mu.l of Grace's medium. The agar is removed by a brief. . .

DETD . . . the expression of the gene of interest a strong promoter of the long terminal repeat (LTR) of the Rouse Sarcoma **Virus** (Cullen, et al., Molecular and Cellular Biology, March 1985, 438-447) plus a fragment isolated from the enhancer of the immediate. . .

DETD Fibroblasts are obtained from a subject by **skin** biopsy. The resulting tissue is placed in tissue-culture medium and separated into small pieces. Small chunks of the tissue are. . .

DETD pMV-7 (Kirschmeier, P. T. et al, DNA, 7:219-25 (1988) flanked by the long terminal repeats of the Moloney murine sarcoma **virus**, is digested with EcoRI and HindIII and subsequently treated with calf intestinal phosphatase. The linear vector is fractionated on agarose. .

.

DETD . . . containing an EcoRI site and the 3' primer further includes a HindIII site. Equal quantities of the Moloney murine sarcoma **virus** linear backbone and the amplified EcoRI and HindIII fragment are added together, in the presence of T4 DNA ligase. The. .

.

DETD . . . with the media from the producer cells. This media is removed and replaced with fresh media. If the titer of **virus** is high, then virtually all fibroblasts will be infected and no selection is required. If the titer is very low,. . .